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(54) **Title:** POLYPEPTIDE HAVING AN ACTIVITY TO SUPPORT PROLIFERATION OR SURVIVAL OF HEMATOPOIETIC STEM CELL AND HEMATOPOIETIC PROGENITOR CELL, AND DNA CODING FOR THE SAME

(57) **Abstract:** A gene encoding a polypeptide having an activity to support proliferation or survival of hematopoietic stem cells or hematopoietic progenitor cells is isolated by comparing expressed genes between cells which support proliferation or survival of hematopoietic stem cells or hematopoietic progenitor cells and cells which do not support the proliferation or survival. Proliferation or survival of hematopoietic stem cells or hematopoietic progenitor cells is supported by using stromal cells in which the isolated gene is expressed or a gene product of the isolated gene.

DESCRIPTION

POLYPEPTIDE HAVING AN ACTIVITY TO SUPPORT PROLIFERATION OR SURVIVAL OF HEMATOPOIETIC STEM CELL AND HEMATOPOIETIC PROGENITOR CELL, AND DNA CODING FOR THE SAME

Background of the Invention

Field of the Invention

The present invention relates to a polypeptide having an activity to support proliferation or survival of hematopoietic stem cells or hematopoietic progenitor cells, a DNA coding the polypeptide, and a pharmaceutical composition comprising the polypeptide as active ingredient.

Description of the Related Art

Fully differentiated mature hematopoietic cells have limited short lives. Homeostasis of the blood is maintained due to supply of the mature blood cells caused by continuous differentiation of hematopoietic progenitor cells. The hematopoietic progenitor cells are giving rise from more undifferentiated hematopoietic stem cells. The hematopoietic stem cells have potential of differentiating into all of the differentiation lineages (totipotency) and have potential of self-renew with retaining the totipotency so as to supply the hematopoietic cells through life. That is, the hematopoietic stem cells are known to generate totipotent stem cells by the self-renew and to

differentiate in parts to a variety of the mature blood cells through the hematopoietic progenitor cells.

This differentiation of the blood cells is regulated by a variety of cytokines. Erythropoietin is known to promote the differentiation of the erythrocytic lineages. G-CSF and thrombopoietin are also known to promote the differentiation of the neutrophils, and the megakaryocytes and the platelet productive cells, respectively. However, a factor required for the self-renew of the hematopoietic stem cell with retaining the totipotency has not been clear. Although SCF/MGF (Williams, D.E., *Cell*, *63*: 167-174, 1990; Zsebo, K.M., *Cell*, *63*: 213-224, 1990), SCGF (WO98/08869), and the like are reported as growth factors for the hematopoietic stem cells, none of them have potency to sufficiently retain the totipotency of the hematopoietic stem cells. Although attempts to culture the hematopoietic stem cells in the presence of combinations of known cytokines, a system for efficient amplification of the hematopoietic stem cells was not realized (Miller, C. L., *Proc. Natl. Acad. Sci. USA*, *94*: 13648-13653, 1997; Yagi, M., *Proc. Natl. Acad. Sci. USA*, *96*: 8126-8131, 1999; Shih, C.C., *Blood*, *94*: 5 1623-1636, 1999).

On the other hand, attempts to allow the hematopoietic stem cells to survive or proliferate without differentiation by using stromal cells which supply an environment suitable for survival or

proliferation of the hematopoietic stem cells were reported (Moore K.A., *Blood*, *89*: 12, 4337-4347, 1997). In addition, WO99/03980 discloses a stromal cell line capable of supporting proliferation or survival of hematopoietic stem cells and hematopoietic progenitor cells, which are established from an AGM (Aorta-Gonad-Mesonephros) region of a fetal mouse.

It is postulated that there should be more peptides that efficiently facilitate hematopoietic stem cell and progenitor cell amplification by themselves or in combination with stromal cells or stimulating factors such as cytokines, in addition to known factors affecting hematopoietic cells.

Summary of the Invention

Since the proliferation or survival of hematopoietic stem cells or hematopoietic progenitor cells *in vitro* can be supported by co-culture of stromal cells and hematopoietic stem cells and hematopoietic progenitor cells, the stromal cells are expected to produce factors supporting the proliferation or survival of hematopoietic stem cells or hematopoietic progenitor cells. An object of the present invention is to provide a factor supporting the proliferation or survival of hematopoietic stem cells or hematopoietic progenitor cells, which is derived from the stromal cells.

The inventor of the present invention has assumed

that the mouse stromal cells produce factors supporting the proliferation or survival of hematopoietic stem cells or hematopoietic progenitor cells, as mentioned above. Attention is given that there are two kinds of stromal cells. One has a ability to support the proliferation or survival of hematopoietic stem cells or hematopoietic progenitor cells (hereafter sometimes referred to as "activity to support hematopoietic stem cells"). The other does not have the activity to support hematopoietic stem cells. The inventor of the present invention has assumed that this difference in the ability is due to the fact that expression of genes encoding the factors is increased in the supporting stromal cells and that the expression is low in non-supporting stromal cells. Thus the inventor think it can be found the factors supporting the proliferation or survival of hematopoietic stem cells or hematopoietic progenitor cells among the genes expressed higher in the supporting cells compared to in the non-supporting cells. In this context, the inventor has identified genes of which expressions are high in AGM-s3-A9 cell line which has the activity to support hematopoietic stem cells, and low or undetected in AGM-s3-A7 cell line which does not have the activity to support hematopoietic stem cells, and has determined the activities to support hematopoietic stem cells, of cells in which these gene groups are highly expressed. As a result, the present

invention has been completed.

That is, the present invention provides the followings.

(1) A DNA coding for a polypeptide of the following (A) or (B):

(A) a polypeptide which comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 19, SEQ ID NO: 23 and SEQ ID NO: 25; or

(B) a polypeptide which comprises an amino acid sequence including deletion, substitution or insertion of one or several amino acids in the amino acid sequence as defined in (A), and which has an activity to support proliferation or survival of hematopoietic stem cells or hematopoietic progenitor cells.

(2) The DNA according to (1), which is a DNA of the following (a) or (b):

(a) a DNA which comprises a nucleotide sequence selected from the group consisting of the nucleotide sequence of nucleotides 1 to 444 of SEQ ID NO: 18, the nucleotide sequence of nucleotides 630 to 1358 of SEQ ID NO: 22, and the nucleotide sequence of nucleotides 132 to 506 of SEQ ID NO: 24; or

(b) a DNA which is hybridizable with a DNA comprising the nucleotide sequence as defined in (a) or a prove prepared from said DNA, under the stringent condition, and which has an activity to support proliferation or survival of hematopoietic stem cells or hematopoietic

progenitor cells.

(3) The DNA according to (2), the stringent condition is 6 x SSC、5 x Denhardt, 0.5% SDS and 68°C (SSC: 3 M NaCl, 0.3 M sodium citrate; 50 x Denhardt: 1% BSA、1% polyvinyl pyrrolidone, 1% Ficoll 400), or 6 x SSC, 5 x Denhardt, 0.5% SDS, 50% formamide and 42°C.

(4) A expression vector which comprises the DNA of any one of (1) to (3) in such a manner that the DNA can be expressed.

(5) A cell into which the DNA of any one of (1) to (3) is introduced in such a manner that the DNA can be expressed.

(6) A polypeptide which is an expression product of the DNA of any one of (1) to (3), the polypeptide having an activity to support proliferation or survival of hematopoietic stem cells or hematopoietic progenitor cells.

(7) The polypeptide according to (6), which comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 19, SEQ ID NO: 23 and SEQ ID NO: 25, or an amino acid sequence including deletion, substitution or insertion of one or several amino acids in the amino acid sequence.

(8) The polypeptide according to (6) or (7), which is modified with one or more modifying agents selected from the group consisting of polyethylene glycol (PEG), dextran, poly(N-vinyl-pyrrolidone),

polypropylene glycol homopoymer, copolymer of polypropylene oxide/ethylene oxide, polyoxyethylated polyol and polyvinyl alcohol.

(9) An monoclonal antibody which binds to the polypeptide of any one of (6) to (8).

(10) A method for supporting proliferation or survival of hematopoietic stem cells or hematopoietic progenitor cells, comprising the step of co-culturing stromal cells in which a DNA coding for a polypeptide of the following (A) or (B) is expressed, with hematopoietic stem cells or progenitor cells,

(A) a polypeptide which comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 23, SEQ ID NO: 25, SEQ ID NO: 27, and SEQ ID NO: 29; or

(B) a polypeptide which comprises an amino acid sequence including deletion, substitution or insertion of one or several amino acids in the amino acid sequence as defined in (A), and which has an activity to support proliferation or survival of hematopoietic stem cells or hematopoietic progenitor cells.

(11) The method according to (10), wherein the DNA is a DNA of the following (a) or (b):

(a) a DNA which comprises a nucleotide sequence selected from the group consisting of the nucleotide sequence of nucleotides 1 to 1671 of SEQ ID NO: 8, the

nucleotide sequence of nucleotides 1 to 1674 of SEQ ID NO: 10, the nucleotide sequence of nucleotides 1 to 366 of SEQ ID NO: 12, the nucleotide sequence of nucleotides 84 to 1121 of SEQ ID NO: 14, the nucleotide sequence of nucleotides 1 to 1035 of SEQ ID NO: 16, the nucleotide sequence of nucleotides 1 to 444 of SEQ ID NO: 18, the nucleotide sequence of nucleotides 1 to 444 of SEQ ID NO: 20, the nucleotide sequence of nucleotides 630 to 1358 of SEQ ID NO: 22, the nucleotide sequence of nucleotides 132 to 506 of SEQ ID NO: 24, the nucleotide sequence of nucleotides 1 to 2487 of SEQ ID NO: 26, and the nucleotide sequence of nucleotides 1 to 2496 of SEQ ID NO: 28; or

(b) a DNA which is hybridizable with a DNA comprising the nucleotide sequence as defined in (a) or a prove prepared from said DNA, under the stringent condition, and which has an activity to support proliferation or survival of hematopoietic stem cells or hematopoietic progenitor cells.

(12) A method for supporting proliferation or survival of hematopoietic stem cells or hematopoietic progenitor cells, comprising the step of culturing hematopoietic stem cells or progenitor cells in the presence of a polypeptide of the following (A) or (B), said polypeptide having an activity to support proliferation or survival of hematopoietic stem cells or hematopoietic progenitor cells when the hematopoietic

stem cells or hematopoietic progenitor cells are cultured in the presence of the polypeptide,

(A) a polypeptide which comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 23, SEQ ID NO: 25, SEQ ID NO: 27, and SEQ ID NO: 29; or

(B) a polypeptide which comprises an amino acid sequence including deletion, substitution or insertion of one or several amino acids in the amino acid sequence as defined in (A), and which has an activity to support proliferation or survival of hematopoietic stem cells or hematopoietic progenitor cells.

(13) A pharmaceutical composition having an effect to support proliferation or survival of hematopoietic stem cells or hematopoietic progenitor cells, which comprises an effective amount of a polypeptide of the following (A) or (B), said polypeptide having an activity to support proliferation or survival of hematopoietic stem cells or hematopoietic progenitor cells when hematopoietic stem cells or hematopoietic progenitor cells are cultured in the presence of the polypeptide,

(A) a polypeptide which comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 23,

SEQ ID NO: 25, SEQ ID NO: 27, and SEQ ID NO: 29; or

(B) a polypeptide which comprises an amino acid sequence including deletion, substitution or insertion of one or several amino acids in the amino acid sequence as defined in (A), and which has an activity to support proliferation or survival of hematopoietic stem cells or hematopoietic progenitor cells.

Terms used in this specification are defined as follows.

A hematopoietic stem cell is defined as a cell having totipotency, that is, ability to differentiate into all the cell lineages of the blood cells, and having a potency of self-renew with retaining the totipotency. A hematopoietic progenitor cell is defined as a cell which can differentiate a single cell lineage of the blood cell or plural cell lineages but cannot differentiate into all of the cell lineages. A stromal cell is defined as a cell which can be co-cultured together with the hematopoietic stem cells to construct a hematopoietic environment simulating *in vivo* hematopoietic environment *in vitro*. Cells derived from any origin can be used as long as the cells can be co-cultured with the hematopoietic cells *in vitro*.

Erythrocyte progenitor cells hardly survive and proliferate in *in vitro* culture environments and rapidly disappear. If the survival and proliferation of the erythrocyte progenitor cells are observed, continuous

production of the erythrocyte progenitor cells is predicted to occur due to the survival and proliferation of the more immature hematopoietic stem cells or the hematopoietic progenitor cells. Therefore, in an assessment system of human hematopoietic stem cells, proliferation of hematopoietic stem cells or immature hematopoietic progenitor cells can be determined by using the survival and proliferation of the erythrocyte progenitor cells (BFU-E, CFU-E, and CFU-E mix) as an index.

Brief Explanation of the Drawings

Fig. 1 shows proliferation statuses of hematopoietic stem cells and hematopoietic progenitor cells determined by a clonogenic assay after co-culture of CD34-positive hematopoietic stem cells with AGM-s3 subclone A9, A7, or D11 cells for two weeks.

Fig. 2 shows proliferation statuses of hematopoietic stem cells and hematopoietic progenitor cells determined by a clonogenic assay after co-culture of CD34-positive hematopoietic stem cells with AGM-s3 subclone A9, A7, or OP9 cells for two weeks.

Fig. 3 shows time course of donor derived lymphoid lineage cells or myeloid lineage cells reconstitution in irradiated recipient mice that received the hematopoietic stem cells co-cultured with stromal cells.

Fig. 4 shows proliferation statuses of hematopoietic

stem cells and hematopoietic progenitor cells determined by a clonogenic assay after co-culture of CD34-positive hematopoietic stem cells with AGM-S3-A9 cells in which a gene SCR-2 is highly expressed (A9/SCR-2), AGM-S3-A9 cells into which a control vector is introduced (A9/pMXIG) or AGM-S3-A9 cells (A9) for two weeks.

Fig. 5 shows proliferation statuses of hematopoietic stem cells and hematopoietic progenitor cells determined by a clonogenic assay after co-culture of CD34-positive hematopoietic stem cells with AGM-S3-A7 cells in which a gene SCR-2 is highly expressed (A7/SCR-2), AGM-S3-A7 cells into which a control vector is introduced (A7/pMXIG) or AGM-S3-A7 cells (A7) for two weeks.

Fig. 6 shows time course of donor derived lymphoid lineage cells or myeloid lineage cells reconstitution in peripheral blood of irradiated recipient mice that received the hematopoietic stem cells co-cultured with AGM-S3-A7 cells in which a gene SCR-3 is highly expressed (A7/SCR-3), AGM-S3-A7 cells into which a control vector is introduced (A7/pMXIG) or AGM-S3-A7 cells.

Fig. 7 shows proliferation statuses of hematopoietic stem cells and hematopoietic progenitor cells determined by a clonogenic assay after co-culture of CD34-positive hematopoietic stem cells with AGM-S3-A9 cells in which a gene SCR-4 is highly expressed (A9/SCR-4), AGM-S3-A9 cells into which a control vector is introduced

(A9/pMXIG) or AGM-S3-A9 cells (A9) for two weeks.

Fig. 8 shows time course of donor derived lymphoid lineage cells or myeloid lineage cells reconstitution in peripheral blood of irradiated recipient mice that received the hematopoietic stem cells co-cultured with AGM-S3-A7 cells in which a gene SCR-5 is highly expressed (A7/SCR-5), AGM-S3-A7 cells into which a control vector is introduced (A7/pMXIG) or AGM-S3-A7 cells.

Fig. 9 shows proliferation statuses of hematopoietic stem cells and hematopoietic progenitor cells determined by a clonogenic assay after co-culture of CD34-positive hematopoietic stem cells with AGM-S3-A9 cells in which a gene SCR-6 is highly expressed (A9/SCR-6), AGM-S3-A9 cells into which a control vector is introduced (A9/pMXIG) or AGM-S3-A9 cells (A9) for two weeks.

Fig. 10 shows proliferation statuses of hematopoietic stem cells and hematopoietic progenitor cells determined by a clonogenic assay after co-culture of CD34-positive hematopoietic stem cells with AGM-S3-A9 cells in which a gene SCR-7 is highly expressed (A9/SCR-7), AGM-S3-A9 cells into which a control vector is introduced (A9/pMXIG) or AGM-S3-A9 cells (A9) for two weeks.

Fig. 11 shows proliferation statuses of hematopoietic stem cells and hematopoietic progenitor cells determined by a clonogenic assay after co-culture

of CD34-positive hematopoietic stem cells with AGM-S3-A9 cells in which a gene SCR-8 is highly expressed (A9/SCR-8), AGM-S3-A9 cells into which a control vector is introduced (A9/pMXIG) or AGM-S3-A9 cells (A9) for two weeks.

Detailed Description of the Invention

Hereafter, the present invention will be described in detail below.

The following genes are those identified as genes of which expressions are high in AGM-s3-A9 cell line which has the activity to support hematopoietic stem cells, and low or undetected in AGM-s3-A7 cell line which does not have the activity to support hematopoietic stem cells, and determined to have the activities to support hematopoietic stem cells, of cells in which these gene groups are highly expressed.

Gene SCR-2

The gene is the same gene as a mouse gene, *Mus musculus* glypican-1 (GPC-1) of a GenBank accession number AF185613.

The nuclotide sequence of the gene from mouse and the amino acid sequence deduced from the nucleotide sequence are shown in SEQ ID NO: 8. Only the amino acid sequence is shown in SEQ ID NO: 9.

The human amino acid sequence of GPC-1 is recorded

in GenBank under an accession number P35052, and the human nucleotide sequence of GPC-1 is recorded in GenBank database under an accession number AX020122. It is predicted that the similar activity is detected in the human gene.

The nucleotide sequence of the gene from human and the amino acid sequence deduced from the nucleotide sequence are shown in SEQ ID NO: 10. Only the amino acid sequence is shown in SEQ ID NO: 11.

Glypican is a major hepran sulfate proteoglycan existing on a cell surface, and have a characteristic structure such as cysteine rich globular domain, short glycosaminoglycan binding domain, glycosylphosphatidyl-inositol membrane binding domain. Six family genes from glypican-1 to glypican-6 have been found (J Biol Chem 1999 Sep 17;274(38):26968-77、Glypican-6, a new member of the glypican family of cell surface heparan sulfate proteoglycans. Veugelers M, De Cat B, Ceulemans H, Bruystens AM, Coomans C, Durr J, Vermeesch J, Marynen P, David G).

With respect to biological activities of GPC-1, there are a number of reports: To regulate growth stimulating activity of heparin binding growth factors (fibroblast growth factor 2 (FGF2), heparin-binding EGF-like growth factor (HB-EGF)) to promote proliferation of cancer cells showing autocrine proliferation by stimulation by the growth factors (J Clin Invest 1998

Nov 1; 102(9):1662-73, The cell-surface heparan sulfate proteoglycan glypican-1 regulates growth factor action in pancreatic carcinoma cells and is overexpressed in human pancreatic cancer., Kleeff J, Ishiwata T, Kumbasar A, Friess H, Buchler MW, Lander AD, Korc M).

To bind HGF (hepatocyte groth factor) to promote reactivity with cytokines, of antigen-specific B cells. To participate in association of a cell with an adhesive molecule to involve in invasion of the cell (J Biol Chem 1998 Aug 28;273(35):22825-32, Heparan sulfate proteoglycans as adhesive and anti-invasive molecules. Syndecans and glypican have distinct functions., Liu W, Litwack ED, Stanley MJ, Langford JK, Lander AD, Sanderson RD). These findings show that GPC-1 involves in activity expression of various cell-stimulating factors. Also, there is a report that expression of the glypican family gene in bone marrow is confirmed (Biochem J 1999 Nov 1;343 Pt 3:663-8, Expression of proteoglycan core proteins in human bone marrow stroma., Schofield KP, Gallagher JT, David G reports, it is not described about effects of GPC-1 on hematopoietic stem cells or hematopoietic progenitor cells.

Gene SCR-3

The gene is the same gene as mouse genes, *Mus musculus* chemokine MMRP2 mRNA of a GenBank accession

number U15209, *Mus musculus* C10-like chemokine mRNA of U19482 and mouse macrophage inflammatory protein-1gamma mRNA of U49513.

The nuclotide sequence of the gene from mouse and the amino acid sequence deduced from the nucleotide sequence are shown in SEQ ID NO: 12. Only the amino acid sequence is shown in SEQ ID NO: 13.

Gene SCR-4

The nuclotide sequence of the gene from mouse and the amino acid sequence deduced from the nucleotide sequence are shown in SEQ ID NO: 14. Only the amino acid sequence is shown in SEQ ID NO: 15.

It has been found that the sequence has a high homology to *Homo sapiens* clone 25077 mRNA of a GenBank accession number AF131820, and that it is considered to be a mouse ortholog. This sequence is described in WO 00/66784.

The nuclotide sequence of the gene from human and the amino acid sequence deduced from the nucleotide sequence are shown in SEQ ID NO: 16. Only the amino acid sequence is shown in SEQ ID NO: 17.

Gene SCR-5

The nuclotide sequence of the gene from mouse and the amino acid sequence deduced from the nucleotide sequence are shown in SEQ ID NO: 18. Only the amino

acid sequence is shown in SEQ ID NO: 19.

It has been found that the sequence has a high homology with *Homo sapiens* esophageal cancer related gene 4 portein (ECRG4) mRNA of a GenBank accession number AF325503, and that it is considered to be a mouse ortholog of AF325503.

The nuclotide sequence of the gene from human and the amino acid sequence deduced from the nucleotide sequence are shown in SEQ ID NO: 20. Only the amino acid sequence is shown in SEQ ID NO: 21.

Gene SCR-6

The nuclotide sequence of the gene from mouse and the amino acid sequence deduced from the nucleotide sequence are shown in SEQ ID NO: 22. Only the amino acid sequence is shown in SEQ ID NO: 23.

Gene SCR-7

The nuclotide sequence of the gene from mouse and the amino acid sequence deduced from the nucleotide sequence are shown in SEQ ID NO: 24. Only the amino acid sequence is shown in SEQ ID NO: 25.

Gene SCR-8

The gene is the same gene as *Mus musculus* mRNA for ADAM23 of a GenBank accession number AB009673.

The nuclotide sequence of the gene from mouse and

the amino acid sequence deduced from the nucleotide sequence are shown in SEQ ID NO: 26. Only the amino acid sequence is shown in SEQ ID NO: 27.

The sequence has a high homology with a sequence described by JP 11155574-A and the sequence described by JP 11155574-A is considered to be a human ortholog.

The nuclotide sequence of the gene from human and the amino acid sequence deduced from the nucleotide sequence are shown in SEQ ID NO: 28. Only the amino acid sequence is shown in SEQ ID NO: 29.

Polypeptides which are products of these genes have an activity to support proliferation or survival of hematopoietic stem cells or hematopoietic progenitor cells. The expression that a polypeptide has an activity to support proliferation or survival of hematopoietic stem cells or hematopoietic progenitor cells means that proliferation or survival of hematopoietic stem cells or hematopoietic progenitor cells is supported in the presence of the polypeptide or in the presence of stroma cells expressing the polypeptide.

Therefore, the present invention provides use of the polypeptides and DNAs encoding the polypeptides and novel polypeptides among the polypeptides and DNAs encoding the novel polypeptides.

A stem cell proliferation-supporting factor which is

a polypeptide encoded by the DNA can be produced by introducing the DNA into a suitable host to prepare a transformant cell, and allowing the DNA to be expressed in the transformant cell.

The DNA may encode the above described factors which have amino acid sequences including substitution, deletion or insertion of one or several amino acids, as long as the activity of the stem cell proliferation-supporting factor to be encoded is not lost. DNAs encoding substantially equivalent polypeptides to this stem cell proliferation-supporting factor can be obtained by modifying the nucleotide sequences so as to include substitution, deletion, insertion, addition, or inversion of amino acid residues in a specific region using site-directed mutagenesis.

The DNAs including the above described mutation can be expressed in appropriate cells and the activity to support hematopoietic stem cells, of the expressed products can be examined, so that the DNAs encoding the polypeptide having functions which are substantially equivalent to this stem cell proliferation-supporting factor are obtained. In addition, the DNAs encoding substantially equivalently active protein as this stem cell proliferation-supporting factor can be obtained by isolating DNAs which hybridize with DNAs including, for example, the nucleotide sequence as described in SEQ ID NO: 8, 10, 12, 14, 16, 18, 20, 22, 24, 26 or 28 from the

cells having the DNA, or probes prepared from these DNAs under the stringent condition; and which encode proteins possessing the activity to support hematopoietic stem cells. The length of the probe is usually 30 to 1000 nucleotides. The stringent condition is, for example, one in which DNAs having homology (determinable with homology search in the compare function of DNASIS version 3.7 (Hitachi Software Engineering)) at not less than 70%, preferably at not less than 80%, are hybridized each other and DNAs having less homology than those are not hybridized each other. The above described stringent condition may be 6 × SSC, 5 × Denhardt, 0.5% SDS, 68°C (SSC; 3 M NaCl, 0.3 M sodium citrate) (50 × Denhardt; 1% BSA, 1% polyvinyl pyrrolidone, 1% Ficoll 400) or 6 × SSC, 5 × Deanhardt, 0.5% SDS, 50% Formamide, 42°C, or the like.

Microorganisms such as *Escherichia coli* and yeast, culture cells derived from animals or plants, and the like are used for host cells for expressing the DNA. Preferably, culture cells derived from mammals are used as the host cells. In the case that prokaryotic cells are used as the host cells, the expression is preferably performed in a condition in which a signal peptide region is replaced with a leader sequence suitable for the prokaryotic cells such as β-lactamase (*bla*), alkaline phosphatase (*phoA*), and outer membrane protein A (*ompA*) and the like, or in a form in which a

methionine residue is added to the N-terminal site of the mature protein.

The introduction of the DNA to the host cell can be carried out by, for example, incorporating the DNA into a vector suitable for the host in an expressible form, and introducing the resultant recombinant vector to the host cell.

Examples of the culture cells derived from mammals include CHO cell, 293 cell, COS7 cell, and the like. Gene expression regulatory sequence such as a promoter to express the DNA may be originated from the gene itself, or may be derived from other genes such as cytomegalovirus promoter and elongation factor 1 promoter and the like.

Examples of a vector for animal culture cells include plasmid vectors, retrovirus vectors, adenovirus vectors (Neering, S.J., *Blood*, *88*: 1147, 1996), herpes virus vectors (Dilloo, D., *Blood*, *89*: 119, 1997), HIV vectors, and the like.

In order to introduce the recombinant vector into culture cells, the conventional methods which are usually employed for transformation of culture cells such as calcium phosphate transfection, the liposome method, the DEAE dextran method, the electroporation method and the microinjection method are employed.

The polypeptides of the present invention also comprise polypeptides having amino acid sequences in

which one or several amino acids are substituted, deleted or inserted in the amino acid sequence represented in SEQ ID NO: 9, 11, 13, 15, 17, 19, 21, 23, 25, 27 or 29, and having activity to support hematopoietic stem cells in addition to the polypeptides having the amino acid sequence represented in SEQ ID NO: 9, 11, 13, 15, 17, 19, 21, 23, 25, 27 or 29. That is, even if mouse and human stem cell proliferation-supporting factors are modified by substitution, deletion, insertion or the like, polypeptides holding essential functions as a stem cell proliferation-supporting factor can be considered to be substantially equivalent to the stem cell proliferation-supporting factor.

These modified stem cell proliferation-supporting factors can be obtained by treating DNA encoding the stem cell proliferation-supporting factor or host cells including the above mentioned DNA with a mutagen, or by mutating the above mentioned DNA so as to substitute, delete, or insert an amino acid at a specific site using site-directed mutagenesis. The residual of the activity to support the hematopoietic stem cells in the obtained mutant polypeptide is confirmed by tranferring hematopoietic stem cells cultured in the presence of the mutant polypeptides into irradiated mice, and monitering peripheral hematological cellularity over time, as in the examples described below.

As for the amino acid deletion, the polypeptide may be a fragment which lacks an amino acid sequence at the N-terminal end and/or the C-terminal end. The fragment lacking the amino acid sequence at the N-terminal end and/or the C-terminal end can be obtained by a usual method, and the hematopoietic stem cell-supporting activity of the fragment can be determined by a similar way to that described with respect to the mutated polypeptide. In particular, if there is a portion predicted as a signal sequence or a transmembrane region in the amino acid sequence, a fragment having the hematopoietic stem cell-supporting activity is predicted by using it as an index. For example, a protein encoded by human SCR-8 is a transmembrane protein of type I passing through the membrane once, and it is therefore predicted that even if it made to be a soluble protein lacking the transmembrane region, it has the activity to support to proliferation or survival of hematopoietic stem cells or hematopoietic progenitor cells. The transmembrane region can be predicted with a known program based on the amino acid sequence. For example, if it is predicted with a program called PSORT II (available through the Internet, URL: http://psort.nibb.ac.jp/index.html), the transmembrane region is amino acids at positions 790 to 806 in SEQ ID NO: 29, and it is predicted that even if a fragment up to position 789, the fragment has activity to support

proliferation or survival of hematopoietic stem cells or hematopoietic progenitor cells.

Since the nucleotide sequences of the above described DNAs have been clarified by the present invention, the DNAs can be also obtained by isolating the corresponding DNAs from mouse or human cDNA or chromosome DNA libraries using PCR in which the oligonucleotides prepared based on these nucleotide sequences are used as primers or using hybridization in which the oligonucleotides prepared based on these nucleotide sequences are used as probes.

In order to complete the present invention, the DNAs of the present invention have been isolated from cDNA library of AGM-s3-A9 cells which are a mouse stromal cell line having the activity to support the hematopoietic stem cells, using SBH (Sequencing By Hybridization) method (Drmanac, S., *Nat. Biotechnol.*, *16*. 54, 1998; Drmanac, R., *Methods. Enzymol.*, *303*, 165, 1999) as described below. The mouse stromal cell lines having the activity to support the hematopoietic stem cells can be obtained using the method disclosed in WO99/03980 or from Cell Bank of Institute of Physical and Chemical Research (RIKEN) or ATCC.

An outline of SBH method will be described below. Probes having eight or nine nucleotides whose sequences are different from each other are prepared. When the nucleotide sequences corresponding to those of the probe

exist in a targeted gene, the probes can hybridize with the gene. The hybridization can be easily detected with utilization of radio isotope- or fluorescence-labelled probes. Each clone in the library is picked up, and blotted on a membrane. Then, the repeated hybridizations are performed with the each of above described probes, so that one can identify the combination of probes that hybridize to each clone. Since the combination of hybridized probes depends on genes, the combination of probes which hybridize to an identical gene is the same. That is, the same gene can be identified as one group (cluster) according to the the combination of the hybridized probes. The number of clones of each gene in the cDNA library can be determined by classifying each clone in the library based on patterns of the hybridized probes and counting the classified clones. Thus, frequency of expression of each gene in the library can be determined.

cDNA libraries are prepared from cells having an activity to support the hematopoietic stem cells and from cells not having the activity. Clustering is performed for the cDNA libraries. Statuses of expressed genes among cells are compared, so that the genes highly expressed with specificity to the supporting cells are selected. The expression statuses of these genes in each of above described cells are further examined by Northern blot analysis, so that genes which are highly

expressed in the cells having the activity to support the hematopoietic stem cells are obtained.

The above mentioned genes are the genes which are highly expressed with specificity to the supporting cells and which are obtained through the above described process. Full-length genes have been cloned from the cDNA library derived from AGM-s3-A9 cell.

Further, in order to determine an ability of gene products to support hematopoiesis, a gene fragment including gene ORF was transferred into stromal cells using a retrovirus vector, and the change in the activity to support the hematopoietic stem cells of the stromal cells was determined. Specifically, after the stromal cells into which the gene was not introduced or into which a control vector was introduced and those into which the gene was introduced were each co-cultured with the mouse hematopoietic stem cells, the hematopoietic cells were transplanted into irradiated mice. The engraftment of the co-cultured hematopoietic cells in recipient mice were examined. As a result, the mice into which the hematopoietic stem cells co-cultured with the gene-introduced cells were transplanted, showed increased chimerism after the transplantation compared with co-culture with the cells into which the gene was not introduced. This result shows that in the gene-expressed stromal cells, an activity to support the proliferation or survival of the hematopoietic stem

cells or the hematopoietic progenitor cells is increased or imparted. As a result, it has become evident that expression of the above described genes has a function to increase the above described activity in the stromal cells or impart the activity to the stromal cells. Therefore, it is revealed that products of the genes affect hematopoietic stem cells or hematopoietic progenitor cells to show an activity to support the survival or the proliferation thereof, or affect stromal cells to show an activity to increase an activity to support the hematopoietic stem cells therein or impart the activity thereto.

The polypeptides of the present invention can be used as a medicine to proliferate or support human hematopoietic stem cells or human hematopoietic progenitor cells when they affect hematopoietic stem cells or hematopoietic progenitor cells to show an activity to support survival or proliferation thereof, in other words, the polypeptides have an activity to support survival or proliferation of hematopoietic stem cells or hematopoietic progenitor cells if the hematopoietic stem cells or the hematopoietic progenitor cells are cultured in the presence of the polypeptides. The pharmaceutical composition can be used for supporting proliferation or survival of human hematopoietic stem cells or human hematopoietic progenitor cells *in vitro*. For hematopoietic stem cell

transplantation therapies such as peripheral blood stem cell transplantation and cord blood stem cell transplantation, a sufficient amount of the hematopoietic stem cells sometimes cannot be collected and the transplantation may not be performed. Even if the enough amount of the stem cells can not be collected, the enough amount of the hematopoietic stem cells can be obtained and transplanted by amplification of the hematopoietic stem cells *in vitro* using this polypeptides. That is, the hematopoietic stem cells can be amplified without differentiation by culturing the hematopoietic stem cells in culture medium including these polypeptides. It may be considered the hematopoietic stem cells are able to be amplified more efficiently with addition of a variety of cytokines to the medium.

When the hematopoietic stem cells or the hematopoietic progenitor cells are cultured in the medium including the polypeptides of the present invention, the hematopoietic stem cells or the hematopoietic progenitor cells used may be isolated one of these cell types alone or may be both of the cell types. In addition, the cells may include at least the hematopoietic stem cells or the hematopoietic progenitor cells, and include other hematopoietic cells. Further, it can be used a fraction containing hematopoietic stem cells or progenitor cells fractionated from the cell

population that contain the hematopoietic stem cells or progenitor cells.

Examples of sources of the hematopoietic stem cells and the hematopoietic progenitor cells in the method of the present invention include a fetal liver, bone marrow, fetal bone marrow, peripheral blood, the peripheral blood from persons whose stem cells are mobilized by administration of cytokines and/or antitumor drugs, cord blood, and the like of mammals such as human and mouse and the like. Any sources may be used as long as the tissue includes the hematopoietic stem cells.

A culture method using petri dishes and flasks for culture may be employed to culture the hematopoietic stem cells or the hematopoietic progenitor cells. The cultivation of the hematopoietic stem cells and/or progenitor cells may be improved by mechanically controlling medium composition, pH, and the like, and using a bioreactor capable of high density cultivation (Schwartz, *Proc. Natl. Acad. Sci. U.S.A.*, *88*: 6760, 1991; Koller, M.R., *Bio/Technology*, *11*: 358, 1993; Koller, M.R., *Blood*, *82*: 378, 1993; Palsson, B.O., *Bio/Technology*, *11*: 368, 1993).

The stromal cells in which DNAs encoding the polypeptide of the present invention can be obtained as described with respect to the expression of the DNAs.

The co-culture of the stromal cells and the hematopoietic cells can be performed simply after the

collection of the bone marrow cells without further separation. Furthermore, co-culture can be performed by separating components such as stromal cells, hematopoietic cells and other cell populations from collected bone marrow and combining them with the hematopoietic cells and stromal cells which are not from the individual from which the bone marrow is cllected. Furthermore, after stromal cells are cultured to grow to the stromal cells, hematopoietic cells can be added to perform co-culture with the hematopoietic stem cells. At this time, cell stimulating factors can added to the culture system of stromal cells to more effectively support proliferation and survival. Concrete examples of the cell stimulating factor include a growth factor which is typically a cytokine such as SCF (stem cell factor), IL-3 (interleukin 3), GM-CSF (granulocyte/macrophage colony-stimulating factor), IL-6 (interleukin 6), TPO (thrombopoietin), G-CSF (granulocyte colony-stimulating factor), TGF-b (transforming growth factor-b), MIP-1a (Davatelis, G., J. Exp. Med. 167: 1939, 1988); a differentiation and proliferation control factor such as hematopoietic hormones such as EPO (erythropoietin), chemokine, Wnt gene product, and notch ligand; and a development control factor.

In addition, when the polypeptide of the present invention affects hematopoietic stem cells or

hematopoietic progenitor cells to show an activity to support survival or proliferation thereof, in other words, the polypeptide has an activity to support survival or proliferation of hematopoietic stem cells or hematopoietic progenitor cells if the hematopoietic stem cells or the hematopoietic progenitor cells are cultured in the presence of the polypeptide, the proliferation and the survival of the hematopoietic stem cells or the hematopoietic progenitor cells can be retained by allowing the recombinant polypeptide of the present invention alone or in combination with the cell stimulating factors to affect hematopoietic stem cells or hematopoietic progenitor cells, without stromal cells. Examples of the cell stimulating factors used in this case are above described cell stimulating factors and the like.

Medium used for the culture is not specially restricted as long as the proliferation or the survival of the hematopoietic stem cells or the hematopoietic progenitor cells is not harmed. Preferable media are, for example, MEM-α medium (GIBCO BRL), SF-02 medium (Sanko Junyaku), Opti-MEM medium (GIBCO BRL), IMDM medium (GIBCO BRL), and PRMI1640 medium (GIBCO BRL). A culture temperature is usually ranging from 25 to 39°C, and preferably ranging from 33 to 39°C. Examples of additives to the medium are fetal bovine serum, human serum, horse serum, insulin, transferrin, lactoferrin,

ethanolamine, sodium selenite, monothiolglycerol, 2-mercaptoethanol, bovine serum albumin, sodium pyruvate, polyethylene glycol, a variety of vitamins, and a variety of amino acids. A concentration of CO_2 is usually ranging from four to six percent, and preferably five percent.

Since the hematopoietic stem cells can differentiate into all the hematopoietic cell lineages, the hematopoietic stem cells can be amplified and differentiated into a specific cell type *in vitro*, and then the specific cells can be transplanted. For example, when erythrocytes are necessary, after the cultivation of the patient's stem cells to amplify them, the hematopoietic cells whose main component is the erythrocyte can be artificially produced using an erythrocyte differentiation induction-promoting factor such as EPO.

The hematopoietic stem cells or the hematopoietic progenitor cells cultured using the polypeptides of the present invention can be used as a graft for blood cell transplantation replacing the conventional bone marrow transplantation or cord blood transplantation. Transplantation of the hematopoietic stem cells is superior to the conventional blood cell transplantation therapy, since the engraftment can last semipermanently.

The transplantation of the hematopoietic stem cells can be employed as therapy for a variety of diseases in

addition to combination therapy with total body X-ray irradiation therapy or advanced chemotherapy for leukemia. For example, when therapy accompanied with myelosuppression as an adverse reaction, such as chemotherapy, radiation therapy, and the like is performed for the patient with solid cancer, the patient can get benefit of early recovery and stronger chemotherapy than the conventional one can be performed to improve the therapeutic effect of the chemotherapy by collecting the bone marrow before the therapy, allowing the hematopoietic stem cells or the hematopoietic progenitor cells to be amplified *in vitro* and returning the amplified cells to the patient after the therapy. In addition, by allowing the hematopoietic stem cells or the hematopoietic progenitor cells obtained according to the present invention to be differentiated into a variety of hematopoietic cells and transplanting these cells into a patient with hypoplasia of a given hematopoietic cells, the patient's deficient status can be improved. In addition, this therapy can improve dyshemopoietic anemia to develop anemia such as aplastic anemia caused by bone marrow hypoplasia. Furthermore, examples of diseases in which the transplantation of the hematopoietic stem cells according to the present invention is effective include immunodeficiency syndrome such as chronic granulomatous disease, duplicated immunodeficiency syndrome, agammaglobulinemia, Wiskott-

Aldrich syndrome, acquired immunodeficiency syndrome (AIDS), and the like, thalassemia, hemolytic anemia due to an enzyme defect, congenital anemia such as sicklemia, Gaucher's disease, lysosomal storage disease such as mucopolysaccharidosis, adrenoleukodegeneracy, a variety of cancers and tumors, and the like.

Transplantation of the hematopoietic stem cells may be performed in the same manner as the conventional bone marrow transplantation or cord blood transplantation other than the differences of the cells used.

The source of the hematopoietic stem cells which may be used for the above described hematopoietic stem cell transplantation is not restricted to the bone marrow, and the above described fetal liver, the fetal bone marrow, the peripheral blood, the peripheral blood with stem cells mobilized by administration of cytokines and/or antitumor drugs, the cord blood, and the like may be used.

The graft may be a composition including buffer solution and the like in addition to the hematopoietic stem cells and the hematopoietic progenitor cells produced by the method according to the present invention.

The hematopoietic stem cells or the hematopoietic progenitor cells produced according to the present invention may be used for *ex vivo* gene therapy. Because of the low frequency of recombination of target genes to

the chromosome because the stem cells are in the resting state, differentiation of the stem cells during the culture period, and the like, the gene therapy to the hematopoietic stem cells has been hard to be established. However, the present invention can amplify the stem cells without differentiation, so that efficacy of gene transfer is expected to be remarkably improved. In this gene therapy, a foreign gene (a gene for therapy) is transferred into the hematopoietic stem cells or the hematopoietic progenitor cells, and then the obtained gene-transferred cells are used. The foreign gene to be transferred is appropriately selected according to disease. Examples of diseases in which the target cells of the gene therapy are the hematopoietic cells include immunodeficiency syndrome such as chronic granulomatous disease, duplicated immunodeficiency syndrome, agammaglobulinemia, Wiskott-Aldrich syndrome, acquired immunodeficiency syndrome (AIDS), and the like, thalassemia, hemolytic anemia due to an enzyme defect, congenital anemia such as sicklemia, Gaucher's disease, lysosomal storage disease such as mucopolysaccharidosis, adrenoleukodegeneracy, a variety of cancers and tumors, and the like.

A usual method used for transfer of a gene into animal cells is employed for the transfer of the gene for the therapy into the hematopoietic stem cells or the hematopoietic progenitor cells. Examples include a

method using a vector for animal cells derived from virus utilized for a gene therapy such as retrovirus vectors such as Moloney mouse leukemia virus, adenovirus vectors, adeno-associated virus (AAV) vectors, herpes simplex virus vectors, and HIV vectors (with respect to a vector for gene therapy, see Verma, I.M., Nature, 389: 239, 1997); calcium phosphate transfection, DEAE-dextran transfection, electroporation, the liposome method, the lipofection method, the microinjection method, and the like. Among them, the method using the retrovirus vector, the adeno-associated virus vector, or the HIV vector is preferable, since permanent expression of a gene is expected due to insertion into the chromosome DNA of a target cell.

For example, the adeno-associated virus (AAV) vector can be prepared as follows. First, a vector plasmid in which a gene for therapy is inserted into ITR (inverted terminal repeat) at both ends of wild-type adeno-associated virus DNA and a helper plasmid for supplementing virus proteins are transfected into 293 cell line. Next, adenovirus as helper virus is infected, so that virus particles including the AAV vector are produced. Alternatively, instead of adenovirus, a plasmid which expresses adenovirus gene having helper function may be transfected. The hematopoietic stem cells or the hematopoietic progenitor cells are infected with the obtained virus particles. Preferably,

appropriate promoter, enhancer, insulator and the like are inserted into the upstream region of the target gene in the vector DNA, so that the expression of the gene is regulated. When a marker gene such as a drug resistant gene is used in addition to the gene for therapy, cells into which the gene for therapy are transferred are easily selected. The gene for therapy may be a sense gene or an antisense gene.

A composition for gene therapy may include a buffer solution and a novel active ingredient and the like in addition to the hematopoietic stem cells or the hematopoietic progenitor cells by the method according to the present invention.

A vector for gene therapy can be produced by incorporating the DNA of the present invention in an expression vector using a usual method. This vector for gene therapy is useful to treat diseases which need survival and proliferation of the human hematopoietic stem cells. That is, the vector for gene therapy is transferred into the hematopoietic stem cells and the cells are cultured *in vitro*, so that the hematopoietic stem cells or the hematopoietic progenitor cells can proliferate dominatingly. The proliferation of hematopoietic stem cells *in vivo* can be caused by returning these hematopoietic stem cells thus treated. The proliferation of hematopoietic stem cells *in vivo* can significantly promoted by introducing this vector

for gene therapy into the body. Alternatively, the bone marrow cells derived from a patient are cultured as it is and this vector for gene therapy is transferred thereto, so that the hematopoietic stem cells or the hematopoietic progenitor cells can be proliferated in a culture system. Alternatively, this vector for gene therapy is transferred into the stromal cells and mesenchaymal stem cells obtained by isolating and culturing stromal cells from the bone marrow, so that the activity to support the hematopoietic stem cells can be added or increased.

As shown in Examples, since it is possible that by introducing the DNA of the present invention into the stromal cells without the activity to support the hematopoietic stem cells, this activity can be imparted, stromal cells having the activity to support the hematopoietic stem cells can be prepared by gene transfer to stromal cells derived from human or mouse. The stromal cells expressing the DNA of the present invention and the hematopoietic stem cells or the hematopoietic progenitor cells are co-cultured, so that the hematopoietic stem cells or the hematopoietic progenitor cells can survive and proliferate so as to be useful for a variety treatment.

Since the hematopoietic stem cells or the hematopoietic progenitor cells can survive and proliferate by expression of the DNA of the present

invention in the stromal cell, an activity to support the hematopoietic stem cells of the stromal cells can be determined using the expression of the DNA of the present invention as an index. The expression of the DNA of the present invention in the stromal cells can be confirmed using an antibody against a polypeptide encoded by the DNA of the present invention. Also, PCR primers can be prepared based on nucleotide sequences, and RNA is prepared from the stromal cells of interest, and RT-PCR is performed, so that the expression of the DNA of the present invention can be confirmed. The antibody will be described below.

The pharmaceutical composition of the present invention can be administered to human. The pharmaceutical composition having an activity to proliferate or to support the human hematopoietic stem cells or the hematopoietic progenitor cells can be produced by mixing medically acceptable diluent, stabilizer, carrier, and/or other additives with the polypeptides of the present invention. At this time, in order to increase the stability of the protein *in vivo*, the polypeptides of the present invention may be modified by a modifying agent. Examples of the modifying agent include polyethylene glycol (PEG), dextran, poly(N-vinyl-pyrrolidone), polypropylene glycol homopolymer, polypropylene oxide/ethylene oxide copolymer, polyoxyethylated polyol, polyvinyl alcohol,

and the like. The modification of the protein with PEG can be performed by, for example, a method in which activated ester derivatives of PEG is reacted with the protein, a method in which aldehyde derivatives at the terminal portion of PEG is reacted with the protein in the presence of a reducing agent, and the like. Japanese Patent Application Laid-Open No. 10-510980 discloses such protein modification in detail.

When the pharmaceutical composition of the present invention is administered to human, recovery from hematological suppression due to an adverse drug reaction of carcinostatics; early recovery of hematopoietic cells at bone marrow transplantation, peripheral blood stem cell transplantation, or cord blood transplantation; and recovery of hematopoietic function at pancytopenia such as aplastic anemia (AA) and myelodysplastic syndrome (MDS) are expected.

The antibodies of the present invention react specifically to the above described polypeptides of the present invention. The antibodies of the present invention may be monoclonal antibodies or polyclonal antibodies as long as they react specifically to the above described polypeptides.

The antibodies of the present invention can be prepared according to usual methods. For example, the antibodies can be prepared either *in vivo* method in which animals are additionally immunized by an antigen

together with adjuvant once or several times at an interval of several weeks or *in vitro* method in which immune cells are isolated and sensitized in an appropriate culture system. Examples of immune cells which can produce the antibodies of the present invention include splenic cells, tonsillar cells, lymph gland cells, and the like.

The whole polypeptide according to the present invention is not necessarily used as an antigen. A part of this polypeptide may be used as an antigen. When the antigen is a short peptide, particularly, a peptide made of about 20 amino acid residues, it may be used by binding it to a carrier protein having high antigenicity such as keyhole lympet hemocyanin or bovine serum albumin using chemical modification and the like. Alternatively, the antigen may be used by covalently binding it to peptide having branching skeleton such as lysine core MAP peptide instead of the carrier protein (Posnett *et al.*, *J. Biol. Chem.*, *263*, 1719-1725, 1988; Lu *et al.*, *Mol. Immunol.*, *28*, 623-630, 1991; Briand *et al.*, *J. Immunol. Methods*, *156*, 255-265, 1992).

Examples of adjuvant include Freund's complete adjuvant, Freund's incomplete adjuvant, aluminum hydroxide gel, and the like. Antigen-given animals are, for example, mouse, rat, rabbit, sheep, goat, chicken, bovine, horse, guinea pig, hamster, and the like. The blood is collected from these animals and the serum is

separated. Then, immunoglobulin is purified from the serum using an ammonium sulfate precipitation method, anion exchange chromatography, protein A chromatography, or protein G chromatography to obtain polyclonal antibodies.

With respect to chicken, antibodies can be purified from an egg. Monoclonal antibodies can be prepared by purification from supernatant of culture of hybridoma cells which are made by fusion of the immune cells sensitized *in vitro*, or immune cells from the above described animals with parent cells capable of cultivation, or ascites from animals which received intraperitoneal administration of hybridoma cells. Examples of parent cells include X63, NS-1, P3U1, X63.653, SP2/O, Y3, SK0-007, GM1500, UC729-6, HM2.0, NP4-1 cell lines, and the like. Preparation may be performed by cultivating the immortalized antibody-forming cells obtained by sensitization *in* vitro, or infection of a proper virus such as EB virus to the immune cells of the above described animals.

In addition to these cell engineering methods, the antibodies can be obtained using gene engineering methods. For example, the antibody gene obtained from the *in vitro* sensitized cells or immune cells derived from the above described animals is amplified by PCR (polymerase chain reaction) and isolated, and the amplified genes are transferred into microorganisms such

as *E. coli* to produce the antibodies. Alternatively, the antibodies may be expressed on surfaces of phages as fused proteins.

By measuring polypeptides *in vivo* using the antibodies of the present invention, the relationship between the polypeptides and pathological status of a variety of diseases can be clarified. Moreover, the antibodies can be used for diagnosis and treatment of diseases, and efficient affinity purification of the polypeptides.

The present invention provides polypeptides having an activity to support survival or proliferation of hematopoietic stem cells or hematopoietic progenitor cells by effecting thereon, or an activity to impart an activity to support the hematopoietic stem cells to stromal cells by effecting thereon, and also provides DNAs encoding thereof. The polypeptides of the present invention can efficiently maintain the proliferation or the survival of the hematopoietic stem cells or the hematopoietic progenitor cells.

Best Mode for Carrying out the Invention

Hereafter, the present invention will be described in detail by reference to examples.

Example 1 Preparation of fragment of gene which is specifically expressed in hematopoietic stem cell-

<u>supporting cells</u>

(I) Preparation of stromal cell line derived from mouse AGM

(1) Isolation of AGM region from fetal mouse

C3H/HeNSLc mice of both genders (purchased from Japan SLC INC.) were kept under a SPF (specific pathogen-free) environment. One or two female mice and one male mouse were placed in the same cage over a night. In the next morning, the female mice in which the existence of a vaginal plug was observed were transferred to other cages and kept. The day when the existence of the vaginal plug was observed was defined to be the 0.5th day of pregnancy. On the 10.5th day of the pregnancy, after mouse was sacrificed by cervical dislocation, fetuses were extirpated. Isolation of AGM regions was performed according to the method by Godin *et al.* (Godin, I., *Proc. Natl. Acad. Sci. U.S.A.*, *92*: 773-777, 1995) and the method by Medvinsky *et al.* (Medvinsky, A.L., *Blood*, *87*: 557-565, 1996). The fetuses were placed in a culture dishes to which PBS(-) (phosphate buffered saline) (produced by Nissui Seiyaku) was added in a volume just sufficient to cover the fetuses. After the AGM regions were carefully excised so as not to include other regions under a stereoscopic microscope, they were put in another 24-well culture dish (Nunc).

(2) Establishment of cell lines derived from AGM

One drop of MEM medium (Sigma) containing 10% FCS (Hyclone) was added to the AGM regions in the 24-well culture dish (Nunc), and AGM regions were cultured in an incubator overnight. The culture was performed in the MEM medium (Sigma) containing 10% FCS (Hyclone) at 37°C, in an atmosphere of 5% CO_2, and at a humidity of 100%. When the cells of the AGM regions adhered to the culture dish due to overnight cultivation, two milliliters of MEM medium containing 10% FCS was further added. Stromal cells began to appear around the AGM region tissue fragment after the continuous cultivation. After one-week cultivation, adhesive cells were separated by trypsin treatment (0.05% trypsin in PBS containing 0.53 mM EDTA (Gibco BRL) at 37°C for three to five minutes). The stromal cells were then washed twice with the medium, and seeded on 6-well culture dish (Nunc). On the next day, the cells which did not adhere to the culture dish and the medium were removed, and then, fresh medium was added. Two weeks after transfer to the 6-well culture dish, cells were γ-ray-irradiated at 900 Rad to eliminate endogenous hematopoietic cells. An attempt of the direct cell cloning by limiting dilution from this culture system was made, but no cell proliferation was observed and the cloning ended in failure. Then, after the number of seeded cells in one well was increased and cells were adapted so as to be able to proliferate from a small number of cells, the cells were cloned by

limiting dilution.

Specifically, the AGM was extirpated and cultured in the same manner as described above. The culture system two weeks after the γ-ray radiation was trypsinized (0.05% trypsin in PBS containing 0.53 mM EDTA at 37°C for three to five minutes) to suspend the cells, and the cells were seeded in a 24-well culture dish at 50 to 100 cells/well. After the culture was continued for three weeks, the cells were seeded in a 96-well culture dish (Nunc) by means of limiting dilution, at 0.3 cells/well. The cells which were grown from the well in which only one cell was seeded were allowed to enlarge culture. As a result, the cells were successfully cloned to obtain fibroblast-like cells and cobble stone-like cells.

A CD34-positive cell fraction derived from the human cord blood was co-cultured with the fibroblast-like cells for two weeks to examine the presence of colony-forming cells during the culture. Colony-forming cells could not be found in the co-culture system with the fibroblast-like cells. Then, the similar examination was performed for seven cell clones showing the cobble-stone-like form. Three clones having an activity to support proliferation of human hematopoietic stem cells were obtained and were named AGM-s1, AGM-s2, and AGM-s3.

(II) Preparation of hematopoietic stem cells from mouse bone marrow

Bone marrow was collected from a femur of C57BL/6-

Ly5.1 pep (eight- to ten-week age, and male) (the gift from Professor K. Nakauchi, University of Tsukuba), and suspended in PBS. After the mouse bone marrow mononuclear cells were concentrated by specific gravity centrifugation according to the usual method (S. Kouzu, Fundamental techniques for immunology, YODOSHA, 1995), the cells were suspended in staining buffer (PBS containing 5% FCS and 0.05% NaN_3), and a hematopoietic stem cell fraction was obtained as follows (Osawa, M. et al., Science 273: 242-245, 1996).

An FITC-conjugated anti-CD34 antibody, a phycoerythrin-conjugated anti-Sca-1 antibody, an allophycocyanin anti-c-Kit antibody (all purchased from Pharmingen) and six biotylated anti-differentiation antigen antibodies (CD45R, CD4, CD8, Gr-1, Ter119, and CD11c, all purchased from Pharmingen) as molecular markers (Lin), were added to a suspension of the bone marrow mononuclear cells and incubated for 20 min on ice to cause reaction. After the cells were washed twice with staining buffer, CD34-negative, Sca-1-positive, c-Kit-positive, and Lin-negative cells were isolated on a cell sorter (FACS Vantage, Becton Dickinson).

(III) Subcloning of mouse stromal cell line and determination of activity to support hematopoietic stem cells of a variety of cell lines

(1) Subcloning of mouse stromal cell line

1) Isolation of AGM-s3 subclone

Stromal cell line AGM-s3 derived from AGM, which was subcultured in MEMα medium (GIBCO BRL) including inactivated 10% FCS (bovine fetal serum, Hyclone), was suspended in PBS containing 5% FCS (PBS-FCS). Clone sorting was performed in a 96-well culture dish (Falcon) at one cell/well using a cell sorter (FACS Vantage; Becton Dickinson). Among cells in the 96 wells, cultures of the cells which grew were expanded, so that thirteen kinds of AGM-s3 subclones were obtained. The activity to support the hematopoietic cells of these AGM-s3 subclones were examined.

2) Isolation of human cord blood CD34-positive stem cell

The human cord blood was collected at normal delivery according to the criteria approved by Ethics committee of Kirin Beer Iyaku Tansaku Kenkyusho. The cord blood was collected using a heparin-added syringe so as not to coagulate. The heparin treated cord blood was overlaid on Lymphoprep (NYCOMED PHARMA), and mononuclear cells were separated by specific gravity centrifugation (at 400G, at room temperature, and for 30 minutes). Erythrocytes contaminated in the mononuclear cell fraction were lyzed by treatment with an ammonium chloride buffer solution (0.83% NH_4Cl-Tris HCl, 20 mM, pH 6.8) at room temperature for two minutes. After the mononuclear cells were washed with PBS-FCS, ten milligrams of human IgG was added thereto and the mixture was allowed to stand on ice for ten minutes.

Then, the cells were further washed with PBS-FCS. Biotinylated antibodies against the antigens specific to the human differentiated blood cells, that is, the antibodies against CD2, CD11c (purified from ATCC hybridoma), CD19 (Pharmingen), CD15, and CD41 (Leinco Technologies Inc.), and Glycophorin A (Cosmo Bio) were added thereto, and the mixture was allowed to stand on ice for 20 min. After washing with PBS-FCS, the cells were suspended in one milliliter of PBS containing 5% FCS, 10 mM EDTA, and 0.05% NaN_3 (PBS-FCS-EDTA-NaN_3). Streptavidin-bound magnetic beads (BioMag. Per Septive Diagnostics) were added thereto, and the mixture was allowed to stand on ice for 40 min. The differentiated blood cells which expressed differentiation antigens were removed using a magnetic separator (Dynal MPC-1 Dynal). An FITC-labeled anti-CD34 antibody (Immunotech S.A., Marseilles, France) was added to the remaining differentiated blood cell antigen-negative cell fraction. After incubation on ice for 20 min., a CD34-positive fraction was recovered using a cell sorter. This cell population was defined as a hematopoietic stem cell population derived from the human cord blood.

3) Co-culture of the human hematopoietic stem cells and AGM-s3 subclone

After 13 kinds of AGM-s3 subclones and stromal cell line MS-5 derived from the mouse bone marrow were each seeded in a 24-well culture dish (Falcon) at 1×10^4

cells/well, and cells were cultured in one milliliter of MEMα medium containing 10% FCS and allowed to grow until the cells covered all over the bottom surfaces of the wells. CD34-positive hematopoietic stem cells derived from the human cord blood were placed on the above described stromal cells at 500 cells/well, and co-cultured in one milliliter of MEMα medium containing 10% FCS. One week after the start of the co-culture, one milliliter of the same medium was further added. Two weeks after the start of the co-culture, the stromal cells and the human blood cells were trypsinized (0.05% trypsin in PBS containing 0.5 mM EDTA (GIBCO BRL) at 37°C; standing for two to five min.) to simultaneously separate them from the culture dish. An activity to support the hematopoietic stem cells was determined with a clonogenic assay.

4) Assessment of proliferation statuses of the hematopoietic stem cells and hematopoietic progenitor cells by clonogenic assay

The cells which proliferated in the above described co-culture system were appropriately diluted, and subjected to one milliliter of methylcellulose culture system to be analyzed. The analysis using the methylcellulose culture system was performed using a 6-well culture dish (Falcon) in MethoCult H4230 (Stem Cell Technologies Inc.) to which 10 ng/ml of human SCF, human IL-3, human IL-6, human G-CSF, and human TPO, and 2

IU/ml of EPO were added. All of a variety of the above described hematopoietic factors were recombinants and pure. After two-week culture, developed colonies were observed under a microscope to count numbers of CFU-GM (granulocyte-macrophage colony-forming unit), BFU-E (erythroid burst forming unit), and CFU-E mix (erythrocyte mixed colony-forming unit).

Fig. 1 shows the result of two-week co-culture of the CD34-positive hematopoietic stem cells and the AGM-s3 subclone A9, A7, or D11. As a result of the co-culture, A9 and D11 subclones among 13 kinds of AGM-s3 subclones supported proliferation of all three series of CFU-GM, BFU-E, and CFU-E mix. Especially, although BFU-E and CFU-E mix, that is, the progenitor cells of erythrocytes were hardly to be supported in usual, their proliferations were observed in the co-culture system with A9 or D11 cells. The results showed that proliferation or maintenance of the hematopoietic stem cells or the hematopoietic progenitor cells occurred in the co-culture with A9 or D11 cells and the progenitor cells of the erythrocyte were continuously supplied. In contrast, although cellular morphology of A7 was similar to that of A9, A7 did not support CFU-GM, BFU-E, and CFU-E mix.

5) Comparison of an activity to support the human hematopoietic stem cells between A9 and a stromal cell line OP9 derived from mouse fetus

Comparison of an activity to support the CD34-positive hematopoietic stem cells derived from the human cord blood between AGM-s3 subclones A9 and A7, and a stromal cell line OP9 derived from mouse fetus (RCB1124, the Cell Development Bank of RIKEN) were performed with CFU-GM, BFU-E, CFU-E and CFU-E mix as indexes, using the above described determination system. Fig. 2 shows the result of the two-week co-culture. In the A7 cell culture system, CFU-GM, BFU-E, and CFU-E were significantly decreased and CFU-E mix was completely disappeared. In contrast, with OP9 cells, a variety of blood cell progenitor cells including CFU-E mix were supported, although the supporting ability was less than that of A9 cells. Therefore, it has been found that OP9 cells possess the activity to support the hematopoietic stem cells.

(2) Assessment of activity to support the hematopoietic stem cells in a variety of cell lines

The above described stromal cell lines (AGM-s3-A9, AGM-s3-A7, and AGM-s3-G1), 3T3Swiss (ATCC), OP9, and NIH3T3 (ATCC) were seeded in a 24-well culture dish (Falcon) at 5 x 10^4 cells/well. The cell lines were cultured in MEMα medium (GIBCO BRL) containing inactivated 10% FCS (bovine fetal serum, Hyclone) for one day and allowed to proliferate until the cells covered all over the bottom surfaces of the wells. Then, the medium was replaced to one milliliter of fresh

medium, thirty cells of the mouse hematopoietic stem cells (derived from C57BL/6-Ly5.1) obtained in the above (II) were placed on this cell layer, and co-culture was started.

On seventh day of the cultivation, the cells were trypsinized (0.05% trypsin in PBS containing 0.5 mM EDTA (GIBCO BRL) at 37°C for two to five minutes) to separate and recover all the cells on the culture dish. The recovered whole cells of each cell line and 200,000 cells of whole bone marrow cells (derived from C57BL/6-Ly5.2 mouse, Charles River) were transplanted into C57BL/6-Ly5.2 mice (eight weeks age and male, Charles River) irradiated with X-ray at 8.5 Gy through the tail vein. After the transplantation, peripheral blood was collected from orbit at intervals, and the ratio in number of cells derived from the C57BL/6-Ly5.1 prep mouse was determined with FACS. The peripheral blood was analyzed according to the usual method (S. Kouzu, Fundamental techniques for immunology, YODOSHA, 1995). Three hundreds and fifty μL of distilled water was added to 50 μL of the peripheral blood, and the mixture was allowed to stand for 30 seconds so as to lyze the erythrocytes. Then, PBS at twice concentrations was added and the mixture was centrifuged to recover white blood cells. After the cells were washed once using the staining buffer (PBS containing 5% FCS and 0.05% NaN_3), anti-CD16 antibody, anti-Ly5.1 (CD45.1) antibody labeled

with FITC, anti-Gr-1 and anti-CD11c antibodies labeled with phycoerythrin, and anti-CD45R (B220) and anti-CD90 (Thy1) antibodies labeled with allophycocyanin (all of these were purchased from Pharmingen) were added. After these cells were allowed to stand for reaction in the ice bath for 30 minites, they were washed with the staining buffer and FACS analysis was performed.

Change in the number of cells capable of reconstitution during the hematopoietic stem cell culture was determined by calculating the proportions of Ly5.1-positive cells in the Gr-1- or CD11c-positive cells (myeloid cells) and Ly5.1-positive cells in the CD90- or CD45R-positive cells (lymphoid cells) in the peripheral blood at intervals after transplantation.

Fig. 3 shows the results. When the cells were co-cultured with AGM-s3-A9 cells, OP9 cells, or 3T3Swiss cells, high chimerism of donor cells were maintained after the transplantation. Therefore, these stromal cells were considered to have a high activity to support the hematopoietic stem cells. In contrast, when the cells were co-cultured with AGM-s3-A7 cells, AGM-s3-G1 cells, or NIH3T3 cells, high chimerism derived from the transplanted cells was not observed. Therefore, these stromal cells were low in an activity to support the hematopoietic stem cells or the hematopoietic progenitor cells.

(IV) Identification of sequences of genes which

specifically express in hematopoietic stem cell-supporting cells

AGM-s3-A9 cells, AGM-s3-A7 cells and OP9 cells were each dissolved in 20 mL of ISOGEN (Nippon gene, Japan) and total RNAs were prepared according to the attachment. Messenger RNAs were prepared from one milligram of the total RNAs according to the protocol of the mRNA purification kit (Amersham Pharmacia, U.S.A.). cDNAs were synthesized from the mRNAs and cDNA libraries (hereinafter, also called as AGM-s3-A9 cDNA, AGM-s3-A7 cDNA and OP9 cDNA, respectively) were constructed using pSPORT1 (GIBCO Lifetech, U.S.A.). A clone harboring a cDNA fragment which highly expresses specifically to AGM-s3-A9 cells or OP9 cells compared with AGM-s3-A7 cells was obtained from the libraries with SBH method (Hyseq, U.S.A.). A nucleotide sequence of the obtained clone was determined using ABI377 DNA sequencer (Perkin Elmer, U.S.A.).

As a result, it has been found that expression of genes comprising nucleotide sequences shown in SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, and SEQ ID NO:7, or parts thereof in AGM-s3-A9 or OP9 cells is higher than that in AGM-s3-A7 cells. These genes were named as SCR-2, SCR-3, SCR-4, SCR-5, SCR-6, SCR-7 and SCR-8, respectively.

Example 2 Cloning of SCR-2 and activity determination

By searching GenBank database for the nucleotide sequence shown in SEQ ID NO: 1 with BLAST, it has been found that SCR-2 is the same gene as a mouse gene, *Mus musculus* glypican-1 (Gpc-1) of an accession number AF185613. The nuclotide sequence of ORF (Open Reading Frame) of SCR-2 and the amino acid sequence deduced from the nucleotide sequence are shown in SEQ ID NO: 8. Only the amino acid sequence is shown in SEQ ID NO: 9.

The human nucleotide sequence of Gpc-1 is recorded in GenBank database under an accession number AX020122. The nucleotide sequence of ORF of AX020122 and the amino acid sequence deduced from the nucleotide sequence are shown in SEQ ID NO: 10. Only the amino acid sequence is shown in SEQ ID NO: 11.

Determination of the activity to support the hematopoietic stem cells or hematopoietic progenitor cells was performed as follows.

(1) Construction of retrovirus vector for expression of mouse SCR-2

Based on the nucleotide sequence of SCR-2 ORF, SCR-2Fsal1 and SCR-2Reco primers having the following nucleotide sequences were prepared, and PCR was performed using OP9 cDNA as a template.

SCR-2Fsal

CCGGTCGACCACCatggaactccggacccgaggctgg (SEQ ID NO: 30)

SCR-2Reco

CCGAATTCttaccgccacctgggcctggctgc (SEQ ID NO: 31)

An amplified fragment was digested with restriction enzymes *Eco*RI and *Sal*I. After electrophoresis, a DNA fragment was purified using JETSORB (Genomed, Germany). The purified DNA fragment was ligated with pMX-IRES-GFP vector digested with *Eco*RI and *Xho*I (gift form Professor T. Kitamura, TOKYO UNIV. INST. OF MEDICAL SCIENCE, Japan). The pMX-IRES-GFP vector is a plasmid obtained by inserting sequences encoding IRES (Internal Ribosome Entry Site) and GFP (Green Fluorescence Protein) into the retrovirus vector pMX. IRES (Internal Ribosome Entry Site) enables ribosome to access to the middle of the mRNA. Therefore, two genes can be expressed from one mRNA by ligation of upward and downward genes separated by IRES in one transcription unit during the construction of an expression vector. With respect to the above-described plasmid, SCR-2 cDNA was inserted in the upward site and GFP (Green Fluorescence Protein) was inserted in the downward site. Thus, the expression of SCR-2 could be monitored by detecting the expression status of GFP using FACS.

The obtained recombinant vector was introduced into *E. coli* DH5α, and was seeded on LB agar medium containing 100 μg/ml of ampicillin, so that independent colonies were formed. After the isolated colony was cultured in 100 mL of LB medium containing 100 μg/ml of

ampicillin, plasmid was purified using QIAGENtip100 (QIAGEN, U.S.A.). The sequence of the inserted gene was determined using a conventional method, so that the sequence was confirmed to be identical to the nucleotide sequence of SCR-2 ORF.

(2) Preparation of stromal cells highly expressing SCR-2

BOSC23 cells were seeded on a collagen type I-coated 60-mm dish (Asahi technoglass) at 2×10^6 cells/dish, and cultured in DMEM medium containing 10% FCS at 37°C, under an atmosphere of 5% CO_2, and at a humidity of 100%. Twelve to 18 hours after the start of the culture, the medium was replaced by two milliliters of OPTI MEM medium (GIBCO BRL).

About 3 μg of plasmid obtained by inserting SCR-2 into the above described pMX-IRES-GFP was added to 18 μl of LIPOFECTAMINE Reagent (GIBCO BRL) diluted with 100 μl of OPTI MEM medium, and the mixture was allowed to stand at room temperature for 30 min. The prepared DNA solution was added to the prepared BOSC23 cell culture solution. After about five hours, two milliliters of DMEM medium containing 20% FCS (GIBCO BRL) was added.

After about 24 hours, the medium was replaced by 4 ml of DMEM containing 10% FCS. Further, after about 48 hours, the culture medium was harvested. After the culture medium was filtrated through 0.45-μm filter, the filtrate was centrifuged at 1,200g for 16 hours and the supernatant was removed to obtain the virus

precipitation.

AGM-s3-A7 or AGM-s3-A9 cells were cultured in one milliliter of MEMα medium containing 10% FCS (GIBCO BRL) on a 24-well culture dish (FALCON) at 1×10^4 cells/well. After 12 to 18 hours, the virus precipitation was suspended in one milliliter of MEMα medium containing 10% FCS, and the stromal cell culture medium was replaced by the virus suspension. Next, POLYBRENE (Sigma, SEQUA-BRENE) was added to be 10 μg/ml. After the culture dish was centrifuged at 700g for 45 minutes, the cells were cultured at 37°C, under an atmosphere of 5% CO_2, and at a humidity of 100%. After 48 hours, the medium was replaced by one milliliter of MEMα medium containing 10% FCS. After 24 hours, the cells were subcultured on a 6-well culture dish (FALCON) and cultured in three milliliters of MEMα medium containing 10% FCS. Forty-eight hours after the subculturing, GFP expression in the stromal cells was detected using a cell sorter (FACSVantage, Becton Dickinson) to indirectly confirm that not less than 80% of cells expressed SCR-2.

Also, the same procedures were repeated by using pMX-IRES-GFP vector instead of the plasmid obtained by inserting SCR-2 into pMX-IRES-GFP to prepare stromal cells into which a control vector was introduced.

(3) Co-culture of human hematopoietic stem cells and stromal cells highly expressing SCR-2, and determination

of proliferation statuses of hematopoietic stem cells and hematopoietic progenitor cells by clonogenic assay

In the same manner as described in (III) (1) 3) to 4) of Example 1, AGM-s3-A9 or AGM-s3-A7 cells in which SCR-2 was highly expressed through retrovirus, AGM-s3-A9 or AGM-s3-A7 cells into which a control vector was introduced, or AGM-s3-A9 or AGM-s3-A7 cells were co-cultured with CD34-positive hematopoietic stem cells derived from human cord blood, and proliferation statuses of hematopoietic stem cells and hematopoietic progenitor cells are determined.

Fig. 4 shows results when the CD34-positive hematopoietic stem cells were co-cultured with AGM-S3-A9 cells in which SCR-2 was highly expressed (A9/SCR-2), AGM-S3-A9 cells into which a control vector was introduced (A9/pMXIG) or AGM-S3-A9 cells (A9) for two weeks. Also, Fig. 5 shows results when the CD34-positive hematopoietic stem cells were co-cultured with AGM-S3-A7 cells in which SCR-2 was highly expressed, AGM-S3-A7 cells into which a control vector was introduced or AGM-S3-A7 cells for two weeks. As a result, by the co-culture with AGM-S3-A9 cells in which SCR-2 was highly expressed or AGM-S3-A7 cells in which SCR-2 was highly expressed, increases of BFU-E and CFU-C were observed. Therefore, it has been revealed that the activity to support hematopoietic stem cells or hematopoietic progenitor cells, of AGM-S3-A9 or AGM-S3-

A7 increases by allowing SCR-2 to be highly expressed. From the results, it has been revealed that a gene product of SCR-2 has an activity to support survival or proliferation of hematopoietic stem cells or hematopoietic progenitor cells or an activity to affect stromal cells to enhance a hematopoietic cell-supporting activity of the stromal cells or impart the activity to the stromal cells.

Example 3 Cloning of SCR-3 and activity determination

By searching GenBank database for the nucleotide sequence shown in SEQ ID NO: 2 with BLAST, it has been found that SCR-3 is the same gene as mouse genes, *Mus musculus* chemokine MMRP2 mRNA of an accession number U15209, *Mus musculus* C10-like chemokine mRNA of U19482 and mouse macrophage inflammatory protein-1gamma mRNA of U49513. The nuclotide sequence of SCR-3 ORF and the amino acid sequence deduced from the nucleotide sequence are shown in SEQ ID NO: 12. Only the amino acid sequence is shown in SEQ ID NO: 13.

Determination of the activity of SCR-3 to support the hematopoietic stem cells or hematopoietic progenitor cells was performed as follows.

(1) Construction of retrovirus vector for expression of mouse SCR-3

Based on the nucleotide sequence of SCR-3 ORF, SCR-3FxhoI and SCR-3Reco primers having the following

nucleotide sequences were prepared, and PCR was performed using AGM-s3-A9 cDNA as a template. An amplified fragment was inserted to the retrovirus vector pMX-IRES-GFP in the same manner as described in (1) of Example 2.

SCR-3FxhoI

ccgCTCGAGccaccATGAAGCCTTTTCATACTGCC (SEQ ID NO: 32)

SCR-3Reco

tccGAATTCttattgtttgtaggtccgtgg (SEQ ID NO: 33)

(2) Preparation of stromal cells highly expressing SCR-3

AGM-s3-A7 cells in which SCR-3 was highly expressed were prepared by using the above retrovirus vector in the same manner as (2) of Example 2.

(3) Determination of activity to support hematopoietic stem cells of stromal cells in which SCR-3 is highly expressed

In the same manner as described in (III) (2) of Example 1, determination of the activity to support hematopoietic stem cells was performed except that AGM-S3-A7 cells, AGM-S3-A7 cells in which SCR-3 was highly expressed through retrovirus, and AGM-S3-A7 cells into which a control vector was introduced were seeded in a 24-well culture dish (Falcon) at 1×10^5 cells/well.

The results are shown in Fig. 6. Hematopoietic cells co-cultured with AGM-s3-A7 cells in which SCR-3 was highly expressed (A7/SCR-3) showed high chimerism in

recipient individuals after the transplantation compared with the parent cell lines or hematopoietic cells co-cultured with the cells into which a control vector was introduced. The high chimerism was observed in myeloid and lymphoid cells two months after the transplantation. Therefore, it is revealed that hematopoietic stem cells and hematopoietic progenitor cells which can reconstitute the hematopoietic system in bodies of irradiated mice have maintained and amplified superiorly to the co-culture with cells into which SCR-3 is not introduced, during the co-culture period. From the results, it is revealed that an activity of stromal cells to support survival or proliferation of hematopoietic stem cells or hematopoietic progenitor cells is increased by high expression of SCR-3. Therefore, it is revealed that a gene product of SCR-3 has an activity to affect hematopoietic stem cells or hematopoietic progenitor cells to support survival or proliferation thereof or an activity to affect stromal cells to enhance a hematopoietic cell-supporting activity of the stromal cells or impart the activity to the stromal cells.

<u>Example 4 Cloning of SCR-4 and activity determination</u>

By searching GenBank database for the nucleotide sequence shown in SEQ ID NO: 3 with BLAST, it has been found that SCR-4 has a high homology to *Homo sapiens*

clone 25077 mRNA of an accession number AF131820, and that SCR-4 is a mouse ortholog. This sequence is described in WO 00/66784.

The nuclotide sequence of ORF of AF131820 and the amino acid sequence deduced from the nucleotide sequence are shown in SEQ ID NO: 16. Only the amino acid sequence is shown in SEQ ID NO: 17.

The nuclotide sequence of ORF of SCR-4 and the amino acid sequence deduced from the nucleotide sequence are shown in SEQ ID NO: 14. Only the amino acid sequence is shown in SEQ ID NO: 15.

Determination of the activity of SCR-4 to support the hematopoietic stem cells or hematopoietic progenitor cells was performed as follows.

(1) Construction of retrovirus vector for expression of human SCR-4

From 3 μg of mRNA derived from fetal liver (CLONETEC, U.S.A.), cDNA was synthesized by using oligo-dT primer and reverse transcriptase (SuperscriptII, GIBCO-BRL). Using the cDNA as a template, the ORF region of human SCR-4 was amplified by PCR with HSCR-4FxhoI and HSCR-4RecoRV primers having the following nucleotide sequences. An amplified fragment was digested with *Xho*I and inserted to the retrovirus vector pMX-IRES-GFP in the same manner as described in (1) of Example 2. For the insertion, the pMX-IRES-GFP was digested with a restriction enzyme *Eco*RI, blunt-ended with KOD DNA

synthase (TOYOBO, Japan) and digested with a restriction enzyme *Xho*I.

HSCR-4FxhoI

CCGCTCGAGCCACCatgttggctgcaaggctggtgt (SEQ ID NO: 34)

HSCR-4RecoRV

CCGGATATCtcatttctttctgttgcctcca (SEQ ID NO: 35)

(2) Preparation of stromal cells highly expressing human SCR-4

AGM-s3-A9 cells in which human SCR-4 was highly expressed were prepared by using the above retrovirus vector in the same manner as (2) of Example 2.

(3) Co-culture of human hematopoietic stem cells and stromal cells highly expressing human SCR-4, and determination of proliferation statuses of hematopoietic stem cells and hematopoietic progenitor cells by clonogenic assay

In the same manner as described in (III) (1) 3) to 4) of Example 1, AGM-s3-A9 cells in which SCR-4 was highly expressed through retrovirus, AGM-s3-A9 cells into which a control vector was introduced, or AGM-s3-A9 cells were co-cultured with CD34-positive hematopoietic stem cells derived from human cord blood, and proliferation statuses of hematopoietic stem cells and hematopoietic progenitor cells are determined.

Fig. 6 shows results when the CD34-positive hematopoietic stem cells were co-cultured with AGM-S3-A9

cells in which human SCR-4 was highly expressed, AGM-S3-A9 cells into which a control vector was introduced or AGM-S3-A9 cells for two weeks. As a result, the co-culture with AGM-S3-A9 cells in which human SCR-4 was highly expressed, increases of BFU-E and CFU-C were observed. Therefore, it has been revealed that the activity to support hematopoietic stem cells or hematopoietic progenitor cells, of AGM-S3-A9 increases by allowing human SCR-4 to be highly expressed. From the results, it has been revealed that human SCR-4 has an activity to support survival or proliferation of hematopoietic stem cells or hematopoietic progenitor cells or an activity to affect stromal cells to impart a hematopoietic cell-supporting activity to the stromal cells.

Example 5 Cloning of SCR-5 and activity determination

In the nucleotide sequence of SEQ ID NO: 4 obtained by the SBH analysis, the presence of ORF was predicted. The nuclotide sequence of ORF and the amino acid sequence deduced from the nucleotide sequence are shown in SEQ ID NO: 18. Only the amino acid sequence is shown in SEQ ID NO: 19.

By searching GenBank database for the nucleotide sequence of SEQ ID NO: 18 with BLAST, it has been found that SCR-5 has a high homology with *Homo sapiens* esophageal cancer related gene 4 portein (ECRG4) mRNA of

an accession number AF325503, and that SCR-5 is a mouse ortholog of AF325503. The nuclotide sequence of ORF of AF325503 and the amino acid sequence deduced from the nucleotide sequence are shown in SEQ ID NO: 20. Only the amino acid sequence is shown in SEQ ID NO: 21.

Determination of the activity of SCR-5 to support the hematopoietic stem cells or hematopoietic progenitor cells was performed as follows.

(1) Construction of retrovirus vector for expression of mouse SCR-5

Based on the nucleotide sequence of SCR-5 ORF, SCR-5FxhoI and SCR-5Rblunt primers having the following nucleotide sequences were prepared for retrovirus cloning, and PCR was performed using DNA having the nucleotide sequence shown in SEQ ID NO: 23 as a template. An amplified fragment was digested with a restriction enzyme *Xho*I and inserted to the retrovirus vector pMX-IRES-GFP in the same manner as described in (1) of Example 2. For the insertion, the pMX-IRES-GFP was digested with a restriction enzyme *Eco*RI, blunt-ended with KOD DNA synthase (TOYOBO, Japan) and digested with a restriction enzyme *Xho*I.

SCR-5FxhoI

ccgCTCGAGccaccatgagcacctcgtctgcgcg (SEQ ID NO: 36)

SCR-5Rblunt

tccGTTAACttaatagtcatcatagttca (SEQ ID NO: 37)

(2) Preparation of stromal cells highly expressing SCR-5

AGM-s3-A7 cells in which SCR-5 was highly expressed were prepared by using the above retrovirus vector in the same manner as (2) of Example 2.

(3) Determination of activity to support hematopoietic stem cells of stromal cells in which SCR-5 is highly expressed

In the same manner as described in (3) of Example 3, determination of the activity to support hematopoietic stem cells was performed.

The results are shown in Fig. 8. Hematopoietic cells co-cultured with AGM-s3-A7 cells in which SCR-5 was highly expressed (A7/SCR-5) showed high chimerism in recipient individuals after the transplantation compared with the parent cell lines or hematopoietic cells co-cultured with the cells into which a control vector was introduced. The high chimerism was observed in myeloid and lymphoid cells two months after the transplantation. Therefore, it is revealed that hematopoietic stem cells and hematopoietic progenitor cells which can reconstitute the hematopoietic system in bodies of irradiated mice have maintained and amplified superiorly to the co-culture with cells into which SCR-5 is not introduced, during the co-culture period. From the results, it is revealed that an activity of stromal cells to support survival or proliferation of hematopoietic stem cells or hematopoietic progenitor

cells is increased by high expression of SCR-5. Therefore, it is revealed that a gene product of SCR-5 has an activity to affect hematopoietic stem cells or hematopoietic progenitor cells to support survival or proliferation thereof or an activity to affect stromal cells to enhance a hematopoietic cell-supporting activity of the stromal cells or impart the activity to the stromal cells.

Example 6 Cloning of SCR-6 and activity determination

Based on the nucleotide sequence of SEQ ID NO: 5, a probe was prepared and AGM-s3-A9 cDNA was screened by hybridization to obtain a gene containing ORF of mouse SCR-6.

AGM-s3-A9 cells (1.4×10^8 cells) were dissolved in 20 mL of ISOGEN (Nippon gene, Japan) and total RNAs were prepared according to the attachment. Messenger RNAs were prepared from one milligram of the total RNAs according to the protocol of the mRNA purification kit (Amersham Pharmacia, U.S.A.). By using SMART cDNA library construction kit (CLONTECH, U.S.A.), cDNA libraries devided to 15 fractions were prepared from the 2 μg of the prepared mRNAs according to the attachment. The libraries contained about 400,000 of independent clones in total. For each fraction, PCR was performed under the following conditions to identify a fraction containing SCR-6 cDNA.

Based on the sequence of a partial fragment of the mouse SCR-6 gene, the following primers were prepared, and PCR was performed with 35 cycles of 94°C, 30 seconds, 55°C, 30 seconds and 72°C, 1 minute, by using each fraction of AGM-s3-A9 cDNA libraries as a template.

SCR-6F

AGCTCATTACTGTATATTTA (SEQ ID NO: 22; 1971-1990)

(SEQ ID NO: 38)

SCR-6R

GCTATATTTCATAAGTCATC (SEQ ID NO: 22; 2330-2349)

(SEQ ID NO: 39)

The PCR product was subjected to 2% agarose gel electrophoresis and a fraction from which the PCR product having the expected size was obtained was identified. For each of two fractions among the positive fractions, 50,000 plaques were seeded on two 15-cm petri dishes and incubated 37°C for 10 hours. Then, plaques of each petri dish were replicated to a sheet of Biodyne nylon filter (Pall, U.S.A.). The replicated nylon filter was subjected to DNA fixation treatment according to the attachment, and screening with ^{32}P-labeled DNA probe was performed.

The probe was prepared as follows. PCR was performed with 35 cycles of 94°C, 30 seconds, 55°C, 30 seconds and 72°C, 1 minute, by using SCR-6F and SCR-6R and the plasmid containing a partial fragment of the

mouse SCR-6 gene as a template. The PCR product was subjected to 2% agarose gel electrophoresis and the amplified fragment was purified by JETSORB. By using 25 ng of the obtained PCR fragment, ^{32}P-labeled DNA probe was prepared with Megaprime labeling kit (Amersham Pharmacia, U.S.A.).

Hybridization and washing were performed with ExpressHybSolution (CLONETECH, U.S.A.) according to the attachment. An X-ray film was exposed to the filter and developed with a Fuji film auto developer to analyze the result. A plaque at a position corresponding to the resultant strongly exposed portion was scraped from the petri dish, and seeded again so that about 200 of plaques should appear on 10-cm petri dish. Screening was again performed according to the above-mentioned method to isolate a single plaque. The obtained clone was transfected to *E. coli* strain BM25.8 according to the attachment of SMART cDNA library construction kit, and the transfected cells were cultured on LB agar medium containing 50 μg/ml ampicilin to form colonies. A single colony of the transfected *E. coli* was inoculated to 3 ml of LB medium containing 50 μg/ml ampicilin and cultured at 30°C overnight. Plasmid was extracted with RPM kit (BIO101, U.S.A.) to obtain about 10 mg of plasmid.

Sequencing the both ends of the inserted fragment with an ABI377 DNA sequencer by using λTriplEx5'LD-

Insert Screening Amplimer (CTCGGGAAGCGCGCCATTGTGTTGGT (SEQ ID NO: 40); CLONTECH, U.S.A.) revealed that it included cDNA containing the nucleotide sequence from nucleotide 1 of SEQ ID NO: 5. The full-length nucleotide sequence was also determined with the ABI377 DNA sequencer. The nuclotide sequence and the amino acid sequence deduced from a nucleotide sequence predicted as ORF in the nucleotide sequence are shown in SEQ ID NO: 22. Only the amino acid sequence is shown in SEQ ID NO: 23.

Determination of the activity of SCR-6 to support the hematopoietic stem cells or hematopoietic progenitor cells was performed as follows.

(1) Construction of retrovirus vector for expression of mouse SCR-6

Based on the nucleotide sequence of SCR-6 ORF, SCR-6FxhoI and SCR-6Reco primers having the following sequences were prepared for retrovirus cloning, and PCR was performed by using DNA having the nucleotide sequence shown in SEQ ID NO: 22 as a template. An amplified fragment was inserted to the retrovirus vector pMX-IRES-GFP in the same manner as described in (1) of Example 2.

SCR-6FxhoI

ccgctcgagccaccATGCGTTTTTGCCTCTTCTC (SEQ ID NO: 41)

SCR-6Reco

cggaattcTTATTGGTTCACTCTGTCTG (SEQ ID NO: 42)

(2) Preparation of stromal cells highly expressing SCR-6

AGM-s3-A9 cells in which SCR-6 was highly expressed were prepared by using the above retrovirus vector in the same manner as (2) of Example 2.

(3) Co-culture of human hematopoietic stem cells and stromal cells highly expressing SCR-6, and determination of proliferation statuses of hematopoietic stem cells and hematopoietic progenitor cells by clonogenic assay

In the same manner as described in (III) (1) 3) to 4) of Example 1, AGM-s3-A9 cells in which SCR-6 was highly expressed through retrovirus, AGM-s3-A9 cells into which a control vector was introduced, or AGM-s3-A9 cells were co-cultured with CD34-positive hematopoietic stem cells derived from human cord blood, and proliferation statuses of hematopoietic stem cells and hematopoietic progenitor cells are determined.

Fig. 9 shows results when the CD34-positive hematopoietic stem cells were co-cultured with AGM-S3-A9 cells in which SCR-6 was highly expressed (A9/SCR-9), AGM-S3-A9 cells into which a control vector was introduced (A9/pMXIG) or AGM-S3-A9 cells (A9) for two weeks. As a result, the co-culture with AGM-S3-A9 cells in which SCR-6 was highly expressed, increases of BFU-E and CFU-C were observed. Therefore, it has been revealed that the activity to support hematopoietic stem cells or hematopoietic progenitor cells, of AGM-S3-A9

increases by allowing SCR-6 to be highly expressed. From the results, it has been revealed that the gene product of SCR-6 has an activity to support survival or proliferation of hematopoietic stem cells or hematopoietic progenitor cells or an activity to affect stromal cells to enhance a hematopoietic cell-supporting activity of the stromal cells or impart the activity to the stromal cells.

Example 7 Cloning of SCR-7 and activity determination

In the nucleotide sequence of SEQ ID NO: 6 obtained by the SBH analysis, the presence of ORF was predicted. The nuclotide sequence of ORF and the amino acid sequence deduced from the nucleotide sequence are shown in SEQ ID NO: 24. Only the amino acid sequence is shown in SEQ ID NO: 25.

Determination of the activity of SCR-7 to support the hematopoietic stem cells or hematopoietic progenitor cells was performed as follows.

(1) Construction of retrovirus vector for expression of mouse SCR-7

Based on the nucleotide sequence of SCR-7 ORF, SCR-7FsalI and SCR-7Reco primers having the following nucleotide sequences were prepared for retrovirus cloning, and PCR was performed using DNA having the nucleotide sequence shown in SEQ ID NO: 24 as a template. An amplified fragment was inserted to the retrovirus

vector pMX-IRES-GFP in the same manner as described in (1) of Example 2.

SCR-7FSalI

acgcgtcgacccaccATGCCCCGCTACGAGTTG (SEQ ID NO: 43)

SCR-7Reco

attGAATTCTCACTTCTTCCTCCTCTTTG (SEQ ID NO: 44)

(2) Preparation of stromal cells highly expressing SCR-7

AGM-s3-A9 cells in which SCR-7 was highly expressed were prepared by using the above retrovirus vector in the same manner as (2) of Example 2.

(3) Co-culture of human hematopoietic stem cells and stromal cells highly expressing SCR-7, and determination of proliferation statuses of hematopoietic stem cells and hematopoietic progenitor cells by clonogenic assay

In the same manner as described in (III) (1) 3) to 4) of Example 1, AGM-s3-A9 cells in which SCR-7 was highly expressed through retrovirus, AGM-s3-A9 cells into which a control vector was introduced, or AGM-s3-A9 cells were co-cultured with CD34-positive hematopoietic stem cells derived from human cord blood, and proliferation statuses of hematopoietic stem cells and hematopoietic progenitor cells are determined.

Fig. 10 shows results when the CD34-positive hematopoietic stem cells were co-cultured with AGM-S3-A9 cells in which SCR-7 was highly expressed (A9/SCR-7), AGM-S3-A9 cells into which a control vector was

introduced (A9/pMXIG) or AGM-S3-A9 cells (A9) for two weeks. As a result, the co-culture with AGM-S3-A9 cells in which SCR-7 was highly expressed, increases of BFU-E and CFU-C were observed. Therefore, it has been revealed that the activity to support hematopoietic stem cells or hematopoietic progenitor cells, of AGM-S3-A9 increases by allowing SCR-7 to be highly expressed. From the results, it has been revealed that the gene product of SCR-7 has an activity to support survival or proliferation of hematopoietic stem cells or hematopoietic progenitor cells or an activity to affect stromal cells to enhance a hematopoietic cell-supporting activity of the stromal cells or impart the activity to the stromal cells.

Example 8 Cloning of SCR-8 and activity determination

By searching GenBank database for the nucleotide sequence shown in SEQ ID NO: 7 with BLAST, it has been found that SCR-8 is the same gene as *Mus musculus* mRNA for ADAM23 of an accession number AB009673. The nuclotide sequence of SCR-8 ORF and the amino acid sequence deduced from the nucleotide sequence are shown in SEQ ID NO: 26. Only the amino acid sequence is shown in SEQ ID NO: 27.

Also, the sequence encoding Human MDC3 protein [*Homo sapiens*] described by JP 11155574-A has a homology of not less than 90% with SCR-8 and, therefore, is a human

ortholog of SCR-8. The nuclotide sequence of this ORF and the amino acid sequence deduced from the nucleotide sequence are shown in SEQ ID NO: 28. Only the amino acid sequence is shown in SEQ ID NO: 29.

Determination of the activity of SCR-8 to support the hematopoietic stem cells or hematopoietic progenitor cells was performed as follows.

(1) Construction of retrovirus vector for expression of mouse SCR-8

Based on the nucleotide sequence of SCR-8 ORF, SCR-8FxhoI and SCR-8Reco primers having the following nucleotide sequences were prepared, and PCR was performed using AGM-s3-A9 cDNA as a template. An amplified fragment was inserted to the retrovirus vector pMX-IRES-GFP in the same manner as described in (1) of Example 2.

SCR-8FxhoI

ccgctcgagccaccATGAAGCCGCCCGGCAGCATC (SEQ ID NO: 45)

SCR-8Reco

cggaattcTCAGATGGGGCCTTGCTGAGT (SEQ ID NO: 46)

(2) Preparation of stromal cells highly expressing SCR-8

AGM-s3-A9 cells in which SCR-8 was highly expressed were prepared by using the above retrovirus vector in the same manner as (2) of Example 2.

(3) Co-culture of human hematopoietic stem cells and stromal cells highly expressing SCR-8, and determination

of proliferation statuses of hematopoietic stem cells and hematopoietic progenitor cells by clonogenic assay

In the same manner as described in (III) (1) 3) to 4) of Example 1, AGM-s3-A9 cells in which SCR-8 was highly expressed through retrovirus, AGM-s3-A9 cells into which a control vector was introduced, or AGM-s3-A9 cells were co-cultured with CD34-positive hematopoietic stem cells derived from human cord blood, and proliferation statuses of hematopoietic stem cells and hematopoietic progenitor cells are determined.

Fig. 11 shows results when the CD34-positive hematopoietic stem cells were co-cultured with AGM-S3-A9 cells in which SCR-8 was highly expressed, AGM-S3-A9 cells into which a control vector was introduced or AGM-S3-A9 cells for two weeks. As a result, the co-culture with AGM-S3-A9 cells in which SCR-8 was highly expressed, increases of BFU-E and CFU-C were observed. Therefore, it has been revealed that the activity to support hematopoietic stem cells or hematopoietic progenitor cells, of AGM-S3-A9 increases by allowing SCR-8 to be highly expressed. From the results, it has been revealed that the gene product of SCR-8 has an activity to support survival or proliferation of hematopoietic stem cells or hematopoietic progenitor cells or an activity to affect stromal cells to enhance a hematopoietic cell-supporting activity of the stromal cells or impart the activity to the stromal cells.

CLAIMS

1. A DNA coding for a polypeptide of the following (A) or (B):

(A) a polypeptide which comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 19, SEQ ID NO: 23 and SEQ ID NO: 25; or

(B) a polypeptide which comprises an amino acid sequence including deletion, substitution or insertion of one or several amino acids in the amino acid sequence as defined in (A), and which has an activity to support proliferation or survival of hematopoietic stem cells or hematopoietic progenitor cells.

2. The DNA according to claim 1, which is a DNA of the following (a) or (b):

(a) a DNA which comprises a nucleotide sequence selected from the group consisting of the nucleotide sequence of nucleotides 1 to 444 of SEQ ID NO: 18, the nucleotide sequence of nucleotides 630 to 1358 of SEQ ID NO: 22, and the nucleotide sequence of nucleotides 132 to 506 of SEQ ID NO: 24; or

(b) a DNA which is hybridizable with a DNA comprising the nucleotide sequence as defined in (a) or a prove prepared from said DNA, under the stringent condition, and which has an activity to support proliferation or survival of hematopoietic stem cells or hematopoietic progenitor cells.

3. The DNA according to claim 2, the stringent

condition is 6 x SSC、5 x Denhardt, 0.5% SDS and 68°C (SSC: 3 M NaCl, 0.3 M sodium citrate; 50 x Denhardt: 1% BSA、1% polyvinyl pyrrolidone, 1% Ficoll 400), or 6 x SSC, 5 x Denhardt, 0.5% SDS, 50% formamide and 42°C.

4. A expression vector which comprises the DNA of any one of claims 1 to 3 in such a manner that the DNA can be expressed.

5. A cell into which the DNA of any one of claims 1 to 3 is introduced in such a manner that the DNA can be expressed.

6. A polypeptide which is an expression product of the DNA of any one of claims 1 to 3, the polypeptide having an activity to support proliferation or survival of hematopoietic stem cells or hematopoietic progenitor cells.

7. The polypeptide according to claim 6, which comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 19, SEQ ID NO: 23 and SEQ ID NO: 25, or an amino acid sequence including deletion, substitution or insertion of one or several amino acids in the amino acid sequence.

8. The polypeptide according to claim 6 or 7, which is modified with one or more modifying agents selected from the group consisting of polyethylene glycol (PEG), dextran, poly(N-vinyl-pyrrolidone), polypropylene glycol homopoymer, copolymer of polypropylene oxide/ethylene oxide, polyoxyethylated

polyol and polyvinyl alcohol.

9. An monoclonal antibody which binds to the polypeptide of any one of claims 6 to 8.

10. A method for supporting proliferation or survival of hematopoietic stem cells or hematopoietic progenitor cells, comprising the step of co-culturing stromal cells in which a DNA coding for a polypeptide of the following (A) or (B) is expressed, with hematopoietic stem cells or progenitor cells,

(A) a polypeptide which comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 23, SEQ ID NO: 25, SEQ ID NO: 27, and SEQ ID NO: 29; or

(B) a polypeptide which comprises an amino acid sequence including deletion, substitution or insertion of one or several amino acids in the amino acid sequence as defined in (A), and which has an activity to support proliferation or survival of hematopoietic stem cells or hematopoietic progenitor cells.

11. The method according to claim 10, wherein the DNA is a DNA of the following (a) or (b):

(a) a DNA which comprises a nucleotide sequence selected from the group consisting of the nucleotide sequence of nucleotides 1 to 1671 of SEQ ID NO: 8, the nucleotide sequence of nucleotides 1 to 1674 of SEQ ID NO: 10, the nucleotide sequence of nucleotides 1 to 366

of SEQ ID NO: 12, the nucleotide sequence of nucleotides 84 to 1121 of SEQ ID NO: 14, the nucleotide sequence of nucleotides 1 to 1035 of SEQ ID NO: 16, the nucleotide sequence of nucleotides 1 to 444 of SEQ ID NO: 18, the nucleotide sequence of nucleotides 1 to 444 of SEQ ID NO: 20, the nucleotide sequence of nucleotides 630 to 1358 of SEQ ID NO: 22, the nucleotide sequence of nucleotides 132 to 506 of SEQ ID NO: 24, the nucleotide sequence of nucleotides 1 to 2487 of SEQ ID NO: 26, and the nucleotide sequence of nucleotides 1 to 2496 of SEQ ID NO: 28; or

(b) a DNA which is hybridizable with a DNA comprising the nucleotide sequence as defined in (a) or a prove prepared from said DNA, under the stringent condition, and which has an activity to support proliferation or survival of hematopoietic stem cells or hematopoietic progenitor cells.

12. A method for supporting proliferation or survival of hematopoietic stem cells or hematopoietic progenitor cells, comprising the step of culturing hematopoietic stem cells or progenitor cells in the presence of a polypeptide of the following (A) or (B), said polypeptide having an activity to support proliferation or survival of hematopoietic stem cells or hematopoietic progenitor cells when the hematopoietic stem cells or hematopoietic progenitor cells are cultured in the presence of the polypeptide,

(A) a polypeptide which comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 23, SEQ ID NO: 25, SEQ ID NO: 27, and SEQ ID NO: 29; or

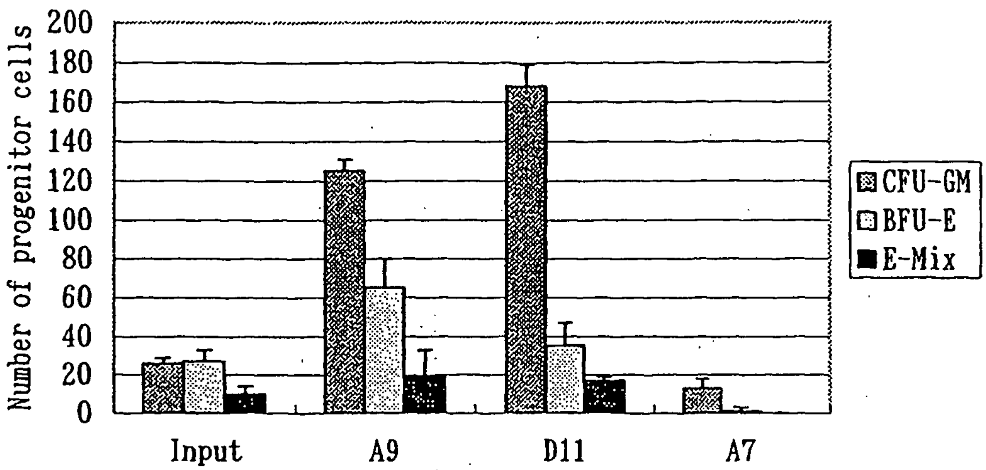
(B) a polypeptide which comprises an amino acid sequence including deletion, substitution or insertion of one or several amino acids in the amino acid sequence as defined in (A), and which has an activity to support proliferation or survival of hematopoietic stem cells or hematopoietic progenitor cells.

13. A pharmaceutical composition having an effect to support proliferation or survival of hematopoietic stem cells or hematopoietic progenitor cells, which comprises an effective amount of a polypeptide of the following (A) or (B), said polypeptide having an activity to support proliferation or survival of hematopoietic stem cells or hematopoietic progenitor cells when hematopoietic stem cells or hematopoietic progenitor cells are cultured in the presence of the polypeptide,

(A) a polypeptide which comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 23, SEQ ID NO: 25, SEQ ID NO: 27, and SEQ ID NO: 29; or

(B) a polypeptide which comprises an amino acid

sequence including deletion, substitution or insertion of one or several amino acids in the amino acid sequence as defined in (A), and which has an activity to support proliferation or survival of hematopoietic stem cells or hematopoietic progenitor cells.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180
160
140
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BFU-E
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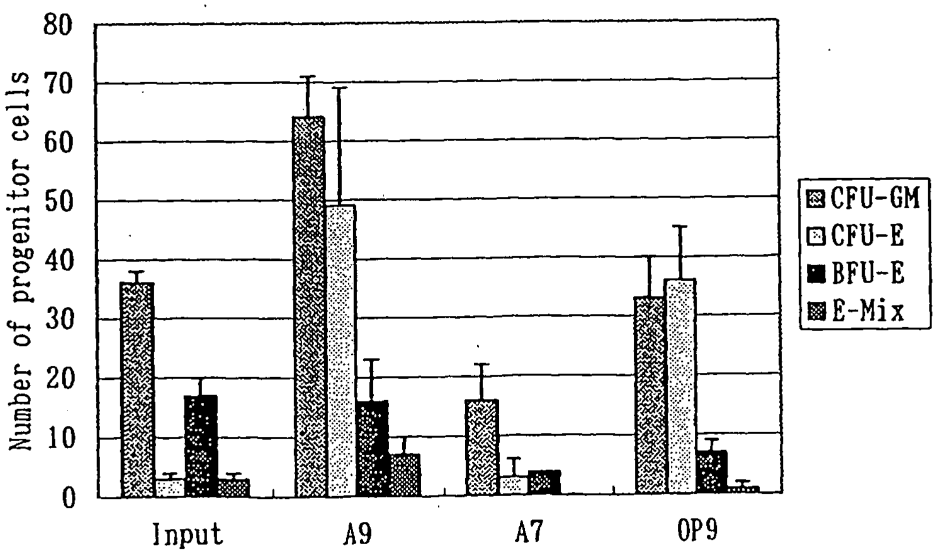
D11
A7

Fig.1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A7
OP9

Fig.2

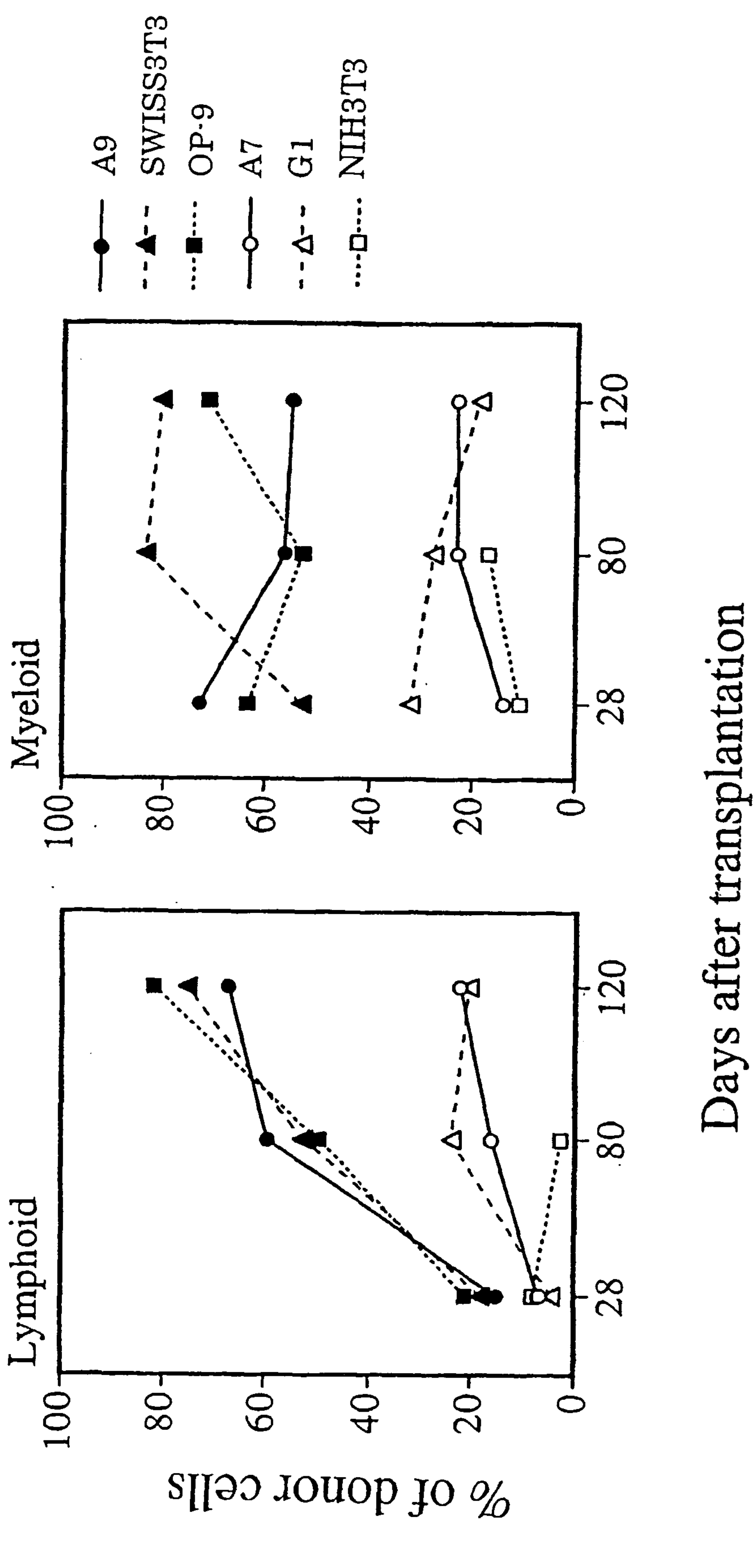
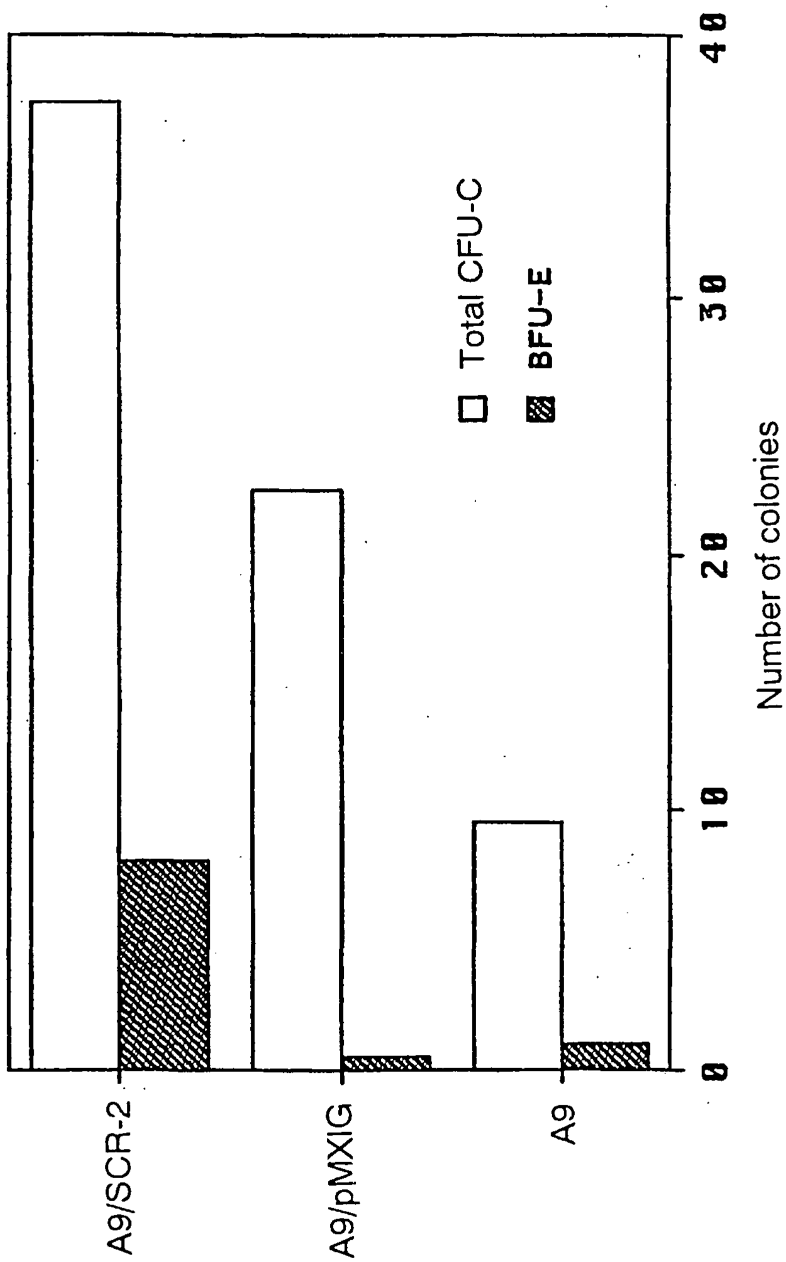

A9
SWISS3T3
OP-9
A7
G1
NIH3T3
Lymphoid
Myeloid
% of donor cells
100
80
60
40
20
0
28
80
120
Days after transplantation

Fig.3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Total CFU-C
BFU-E
0
10
20
30
40
Number of colonies

Fig. 4

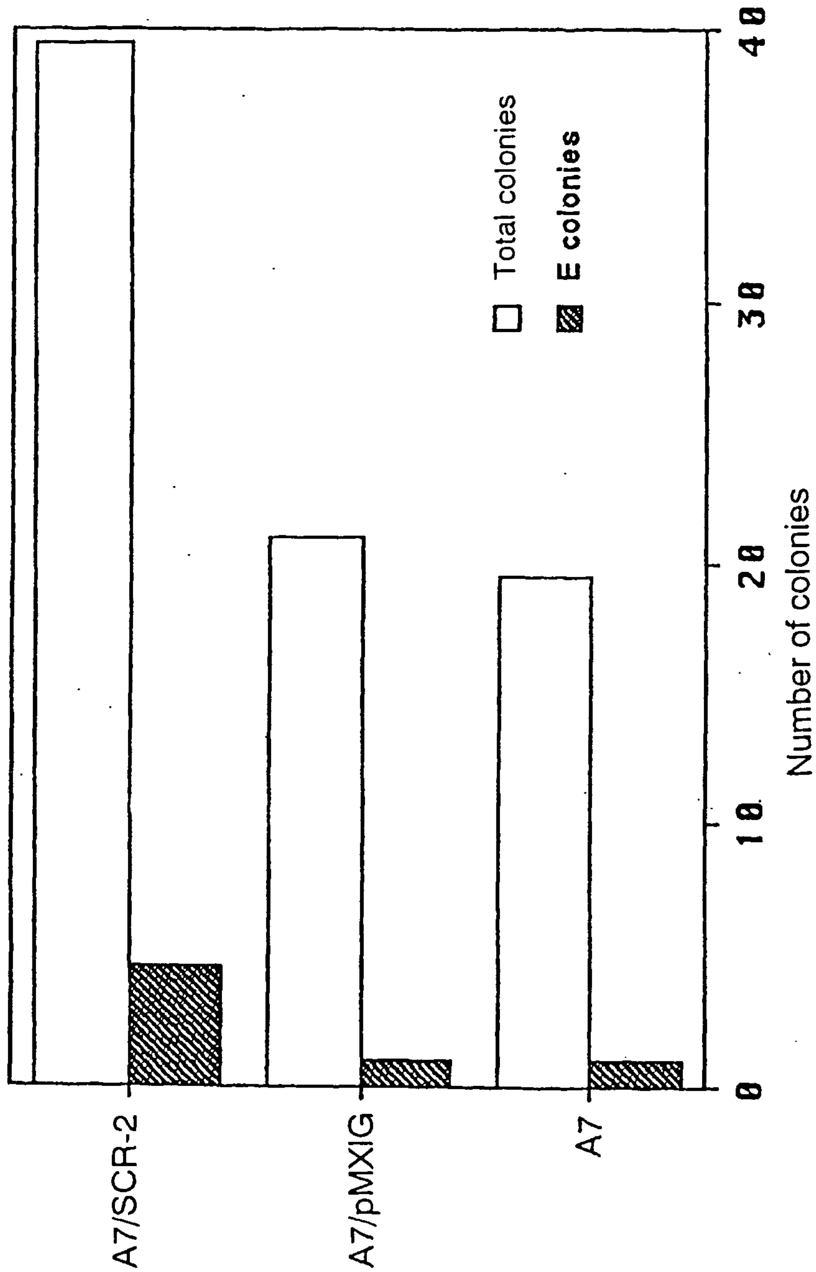

Total colonies
E colonies
A7/SCR-2
A7/pMXIG
A7
0
10
20
30
40
Number of colonies

Fig. 5

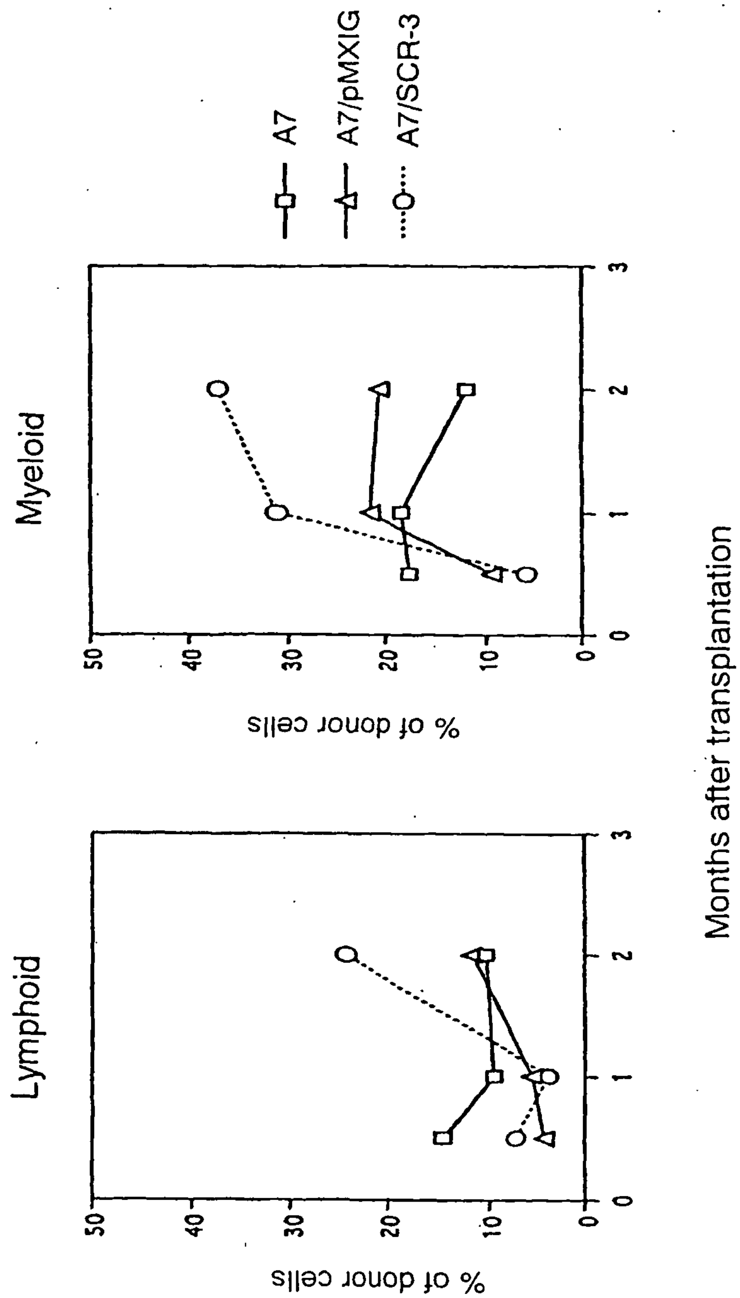

Lymphoid
Myeloid
% of donor cells
% of donor cells
Months after transplantation
A7
A7/pMXIG
A7/SCR-3
0
10
20
30
40
50
0
1
2
3

Fig. 6

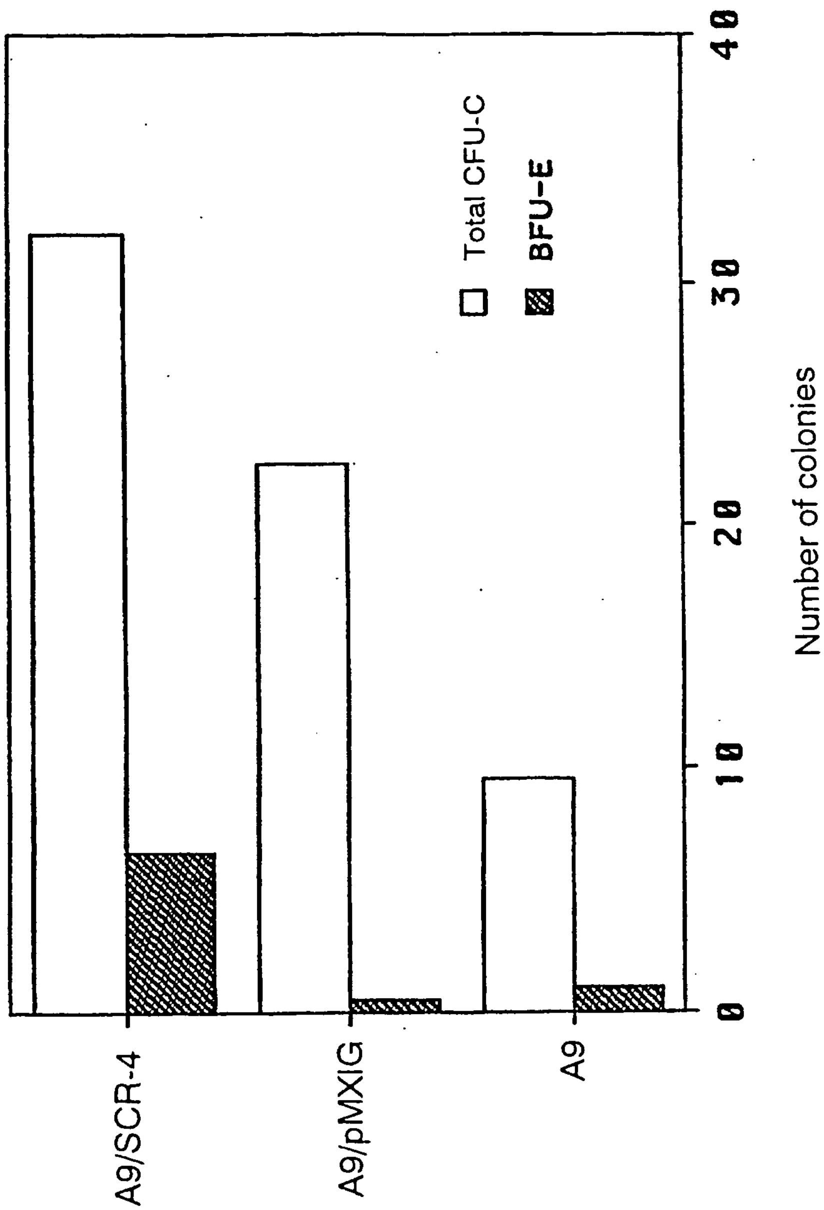

Total CFU-C
BFU-E
A9/SCR-4
A9/pMXIG
A9
0
10
20
30
40
Number of colonies

Fig. 7

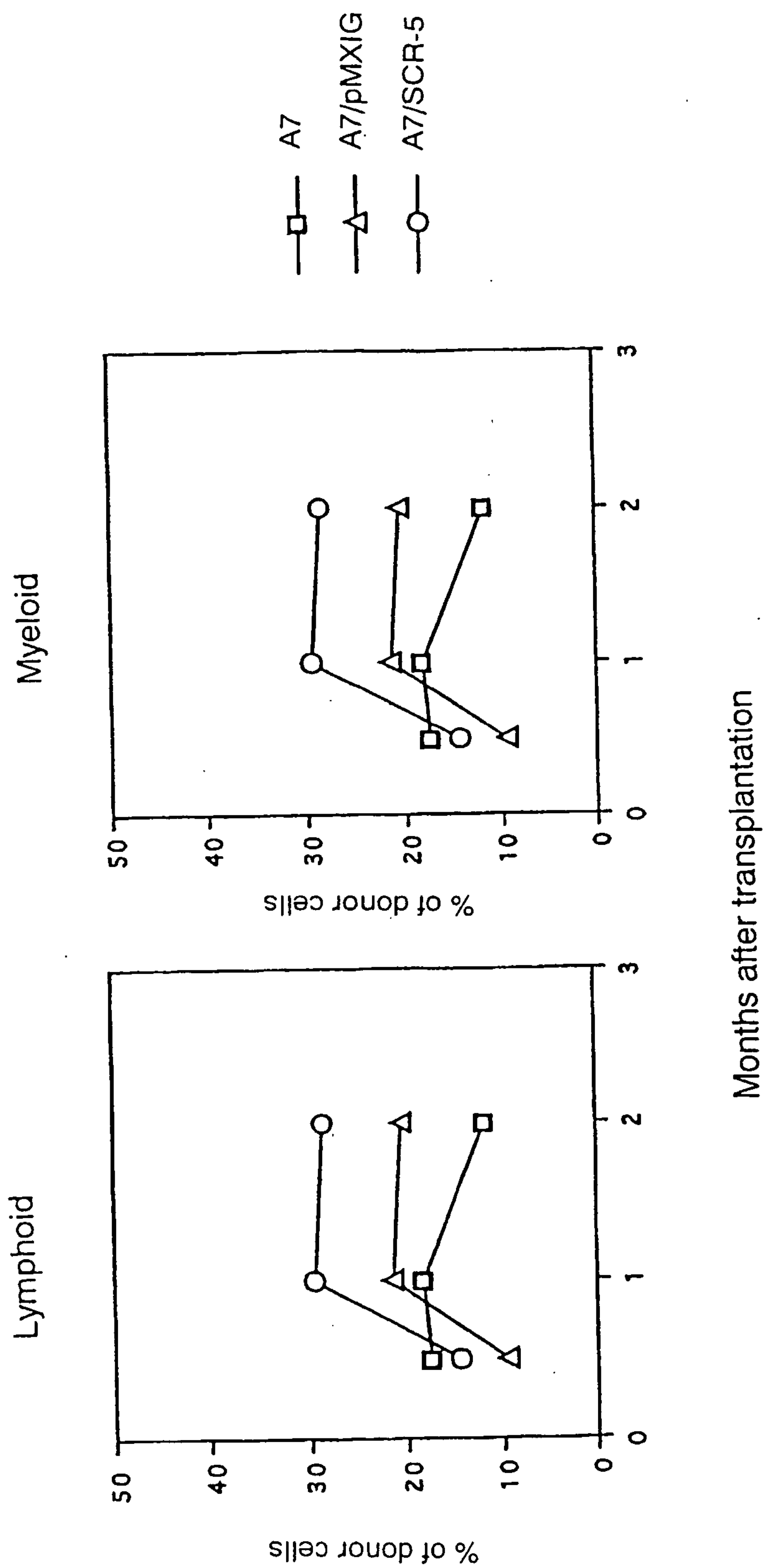
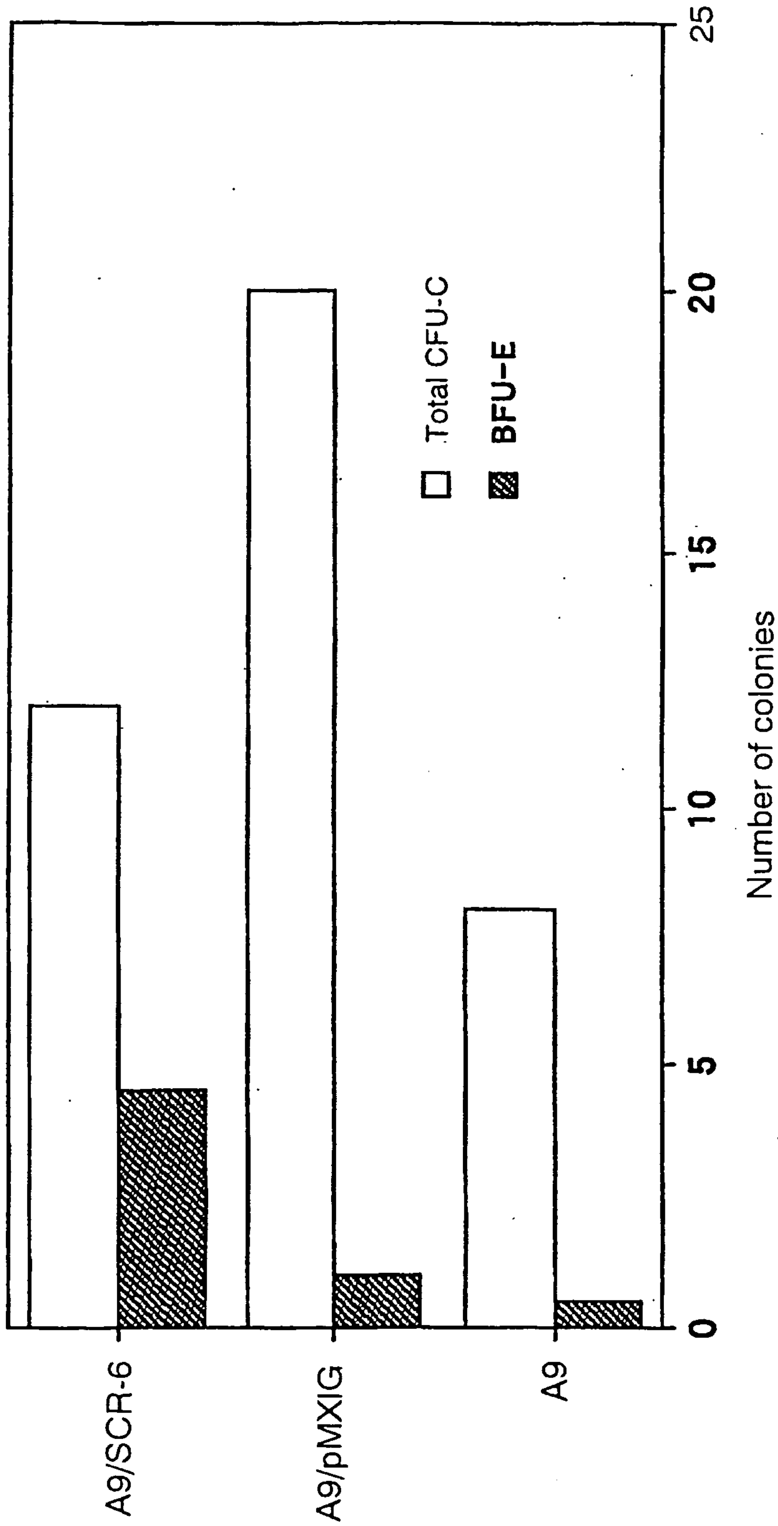

A7
A7/pMXIG
A7/SCR-5
Lymphoid
Myeloid
% of donor cells
% of donor cells
Months after transplantation
0
10
20
30
40
50
0
1
2
3

Fig. 8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A9/pMXIG
A9
Total CFU-C
BFU-E
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5
10
15
20
25
Number of colonies

Fig. 9

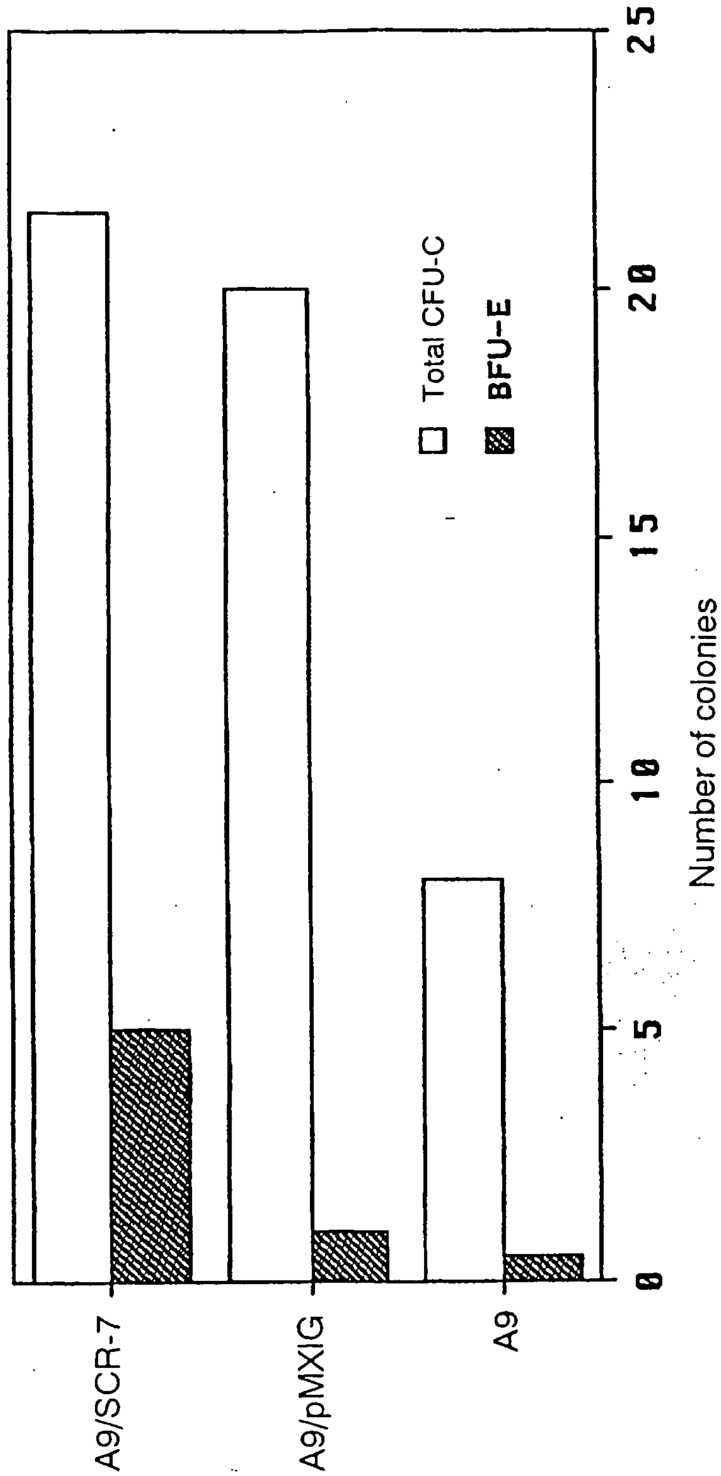
Total CFU-C
BFU-E
A9/SCR-7
A9/pMXIG
A9
0
5
10
15
20
25
Number of colonies

Fig. 10

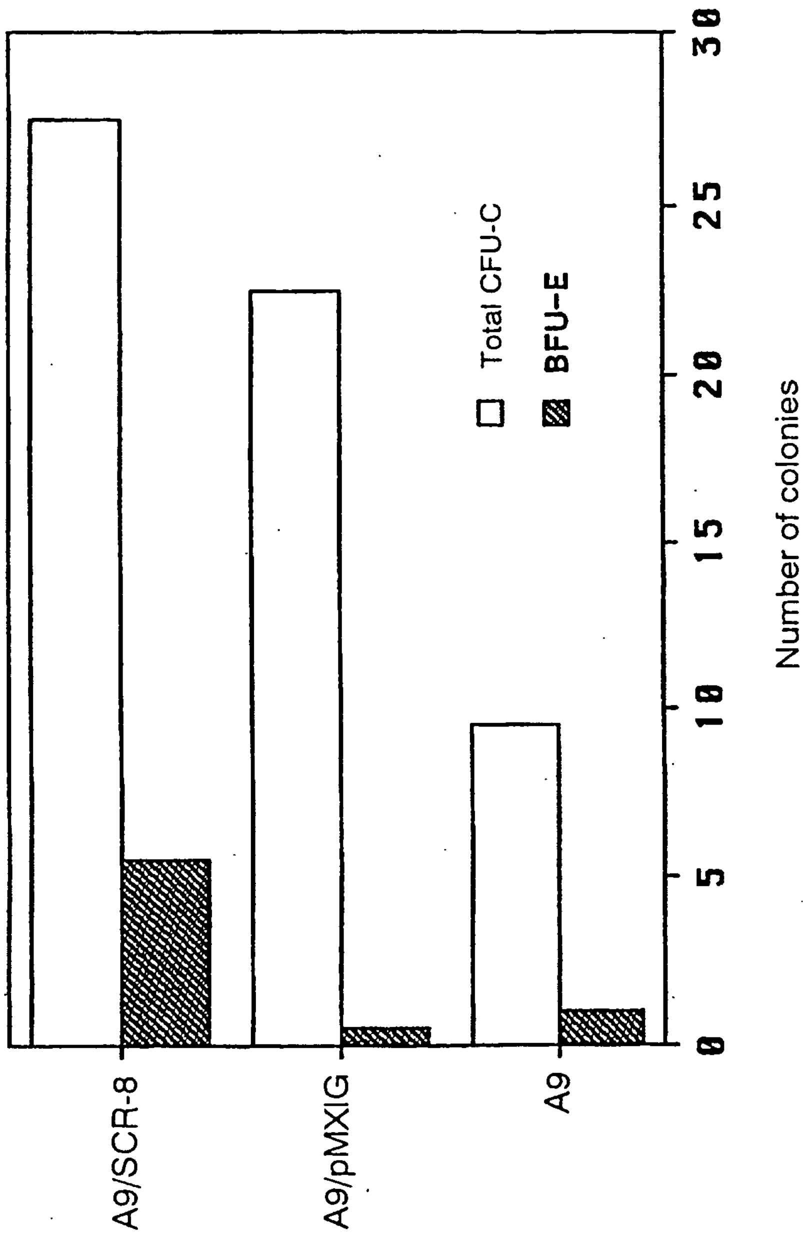
A9/SCR-8
A9/pMXIG
A9
Total CFU-C
BFU-E
0
5
10
15
20
25
30
Number of colonies

Fig. 11

SEQUENCE LISTING

<110> KIRIN BEER KABUSHIKI KAISHA

<120> POLYPEPTIDE HAVING AN ACTIVITY TO SUPPORT PROLIFERATION OR SURVIVAL OF HEMATOPOIETIC STEM CELL AND HEMATOPOIETIC PROGENITOR CELL, AND DNA CODING FOR THE SAME

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cactctgttc ctatttacta agagggtagg ggtctcctta atatcatact tcattgttaa 1320

taagtcaatg cttgttatgt ttcttggctg ttgtttttgt gcattaaaaa ctcaaaattg 1380

gaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa 1420

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<211> 763
<212> DNA
<213> Mus musculus

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cgtacaatag aatccctgat ggaccgagga gccatagtga ggaacttgga aagcctgggt 180

gagcgtgcgc tcccctacag gatctcgagt cacagccagc agcacagccg aggagggtat 240

ttcctggtgg atttttatgc tccgacaagt gctgtggaga acatactgga acacttggcg 300

cgagacattg acgtggttag accaaatatt gtgaaacacc ctctgaccca ggaagtaaaa 360

gagtgtgacg gcatagtccc agtcccactt gaagaaaaac tgtattcaac aaagaggagg 420

aagaagtgag aagattcacc agattctggc cttatattta atcctaaggg cactatgggt 480

gctgctaggt tgttgtctag gatactttag cccatgacca ttttgctgca ggaggtagaa 540

actgctggcc gagacctgcc ctgatgtctc tgctgagatt tcatcccact tgtggggttt 600

gtcgggagtg gggtgttcca cagtaccact gtagcgtttc caagagcaaa atgtttgtca 660

ttcacacttg gttgtcttgc aagcctatat ggaacactgg gagcagagta ataaacatga 720

ctttatcaac actggaaaaa aaaaaaaaaa aaaaaaaaaa aaa 763

<210> 7
<211> 1300
<212> DNA
<213> Mus musculus

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ggcatgggct cagcttggag gaaagtcaga ggaaattcag ataacttggt atctcttcct 180

gtcgttgcaa tgtttcgggg tccacttcac tatgagatac caagcagctg ccaacctcac 240

catactcatt tcgttacaat ttctgaggca ccgtggtgac ttgatccgac atacgaccac 300

gtcagttaca aaccagatct ttatggttaa cttttgaaca tttcacaaac aacattgtaa 360

atgtgcgatg ttatgtttta aatcagacca cagtggtccc caaatattat gtacatatga 420

caaatgtcag tgtaactttt tgttacactg acagtttcat aggtaaacaa acctacgctc 480

caatgttaaa ttatgcttgt gtatgtaaaa tacacaagca ttgggctatg tgtgtacgga 540

catgagggta gtgcaatcgt actgtacgaa atgggtcaga atcattttca gtggtgttag 600

gttatgtagt ttcagactcc atgctgcatt ttctcttgca catgccatcc atttgcttat 660

tttggagtgt gagtattcct tcttattaat ttgaattcaa agcacaagcc tcccattgtt 720

caacattacc caacaagagt gtccagtgat gaccgagtta tctcacctgc tatactttta 780

ctgcaataat taatgacacc tggatgagga ggcgtgcgct gacttcattg ttcacccggg 840

atagtgcatg agcccactga attagagctg cttctaccag caaaagtgag cagtacacat 900

aggtgcatgt ttgaaacatg aatcacatag agctatggag ttttgccaag tgatgtgttt 960

tctttttctt ttttcttttt ttttcttttt cttctttttt ttccttttct tcttcttctt 1020

cttttttttt ttttttacta tgcaaagatg ggaaatgcac aaacttccaa gacatgtctg 1080

aagaacttta caatacttga attttttctt taatcatccc atcacattta tggcattgat 1140

gcttccattg tatttttctt ttgtcccttc aacttcaatg gtttgtaatt tcaatgcaca 1200

acctaacttt tgtttgcagt aacttccaat cctattggct gcctggaacg gagattctgt 1260

catcctacac gcatctttta gttgactgtg cataaaagtt 1300

<210> 8
<211> 1674
<212> DNA
<213> Mus musculus

<220>
<221> CDS
<222> (1)..(1671)

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Met Glu Leu Arg Thr Arg Gly Trp Trp Leu Leu Cys Ala Ala Ala Ala
1 5 10 15

ctg gtc gtc tgc gcc cgc ggg gac ccc gcc agc aag agc cgg agc tgc 96
Leu Val Val Cys Ala Arg Gly Asp Pro Ala Ser Lys Ser Arg Ser Cys
20 25 30

agc gaa gtc cgc cag atc tac ggg gct aag ggc ttt agc ctg agc gat 144
Ser Glu Val Arg Gln Ile Tyr Gly Ala Lys Gly Phe Ser Leu Ser Asp
35 40 45

gtg ccc cag gca gag atc tcg ggt gag cac ctg cgg atc tgc ccc cag 192
Val Pro Gln Ala Glu Ile Ser Gly Glu His Leu Arg Ile Cys Pro Gln
50 55 60

ggc tac act tgc tgt acc agt gag atg gag gag aat ttg gcc aac cac 240
Gly Tyr Thr Cys Cys Thr Ser Glu Met Glu Glu Asn Leu Ala Asn His
65 70 75 80

agc cga atg gag ctg gag agc gca ctc cat gac agc agc cgc gcc ctg 288
Ser Arg Met Glu Leu Glu Ser Ala Leu His Asp Ser Ser Arg Ala Leu
85 90 95

cag gcc aca ctg gcc acc cag ctg cat ggc atc gat gac cac ttc cag 336
Gln Ala Thr Leu Ala Thr Gln Leu His Gly Ile Asp Asp His Phe Gln

100 105 110

cgc ctg ctg aat gac tcg gag cgc aca ctg cag gag gct ttc cct ggg 384
Arg Leu Leu Asn Asp Ser Glu Arg Thr Leu Gln Glu Ala Phe Pro Gly
115 120 125

gcc ttt ggg gac ctg tat acg cag aac act cgt gcc ttc cgg gac cta 432
Ala Phe Gly Asp Leu Tyr Thr Gln Asn Thr Arg Ala Phe Arg Asp Leu
130 135 140

tat gtt gag ctg cgc ctc tac tac cgt ggg gcc aac ctg cac ctt gag 480
Tyr Val Glu Leu Arg Leu Tyr Tyr Arg Gly Ala Asn Leu His Leu Glu
145 150 155 160

gag acg ctg gcc gag ttc tgg gca cgg ctg ctg gag cgc ctc ttc aag 528
Glu Thr Leu Ala Glu Phe Trp Ala Arg Leu Leu Glu Arg Leu Phe Lys
165 170 175

cag ctg cac ccc cag ctg ctg cct gat gac tac ctg gac tgc ctg ggc 576
Gln Leu His Pro Gln Leu Leu Pro Asp Asp Tyr Leu Asp Cys Leu Gly
180 185 190

aag cag gcg gag gca ctg cgg ccg ttt gga gat gcc cct cga gaa ctg 624
Lys Gln Ala Glu Ala Leu Arg Pro Phe Gly Asp Ala Pro Arg Glu Leu
195 200 205

cgc ctg cgg gcc acc cgt gcc ttt gtg gct gca cgt tcc ttt gtg cag 672
Arg Leu Arg Ala Thr Arg Ala Phe Val Ala Ala Arg Ser Phe Val Gln
210 215 220

ggc ctg ggt gtg gcc agt gat gta gtc cgg aag gtg gcc cag gta cct 720
Gly Leu Gly Val Ala Ser Asp Val Val Arg Lys Val Ala Gln Val Pro
225 230 235 240

ctg gcc cca gaa tgt tct cgg gcc atc atg aag ttg gtc tac tgt gct 768
Leu Ala Pro Glu Cys Ser Arg Ala Ile Met Lys Leu Val Tyr Cys Ala
245 250 255

cat tgc cgg gga gtc ccg ggc gcc cgg ccc tgc ccc gac tat tgc cga 816
His Cys Arg Gly Val Pro Gly Ala Arg Pro Cys Pro Asp Tyr Cys Arg
260 265 270

aat gtg ctc aaa ggc tgc ctt gcc aac cag gcc gac ctg gat gcc gag 864
Asn Val Leu Lys Gly Cys Leu Ala Asn Gln Ala Asp Leu Asp Ala Glu
275 280 285

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tgg agg aac ctc ctg gac tcc atg gtg ctc atc act gac aag ttc tgg       912
Trp Arg Asn Leu Leu Asp Ser Met Val Leu Ile Thr Asp Lys Phe Trp
    290                 295                 300

ggc ccg tcg ggt gcg gag agt gtc att ggc ggt gtg cac gtg tgg ctg       960
Gly Pro Ser Gly Ala Glu Ser Val Ile Gly Gly Val His Val Trp Leu
305                 310                 315                 320

gcg gag gcc atc aac gcc ctc cag gac aac aag gac aca ctc aca gct      1008
Ala Glu Ala Ile Asn Ala Leu Gln Asp Asn Lys Asp Thr Leu Thr Ala
                325                 330                 335

aag gtc atc cag gcc tgt gga aac ccc aag gtc aat ccc cac ggc tct      1056
Lys Val Ile Gln Ala Cys Gly Asn Pro Lys Val Asn Pro His Gly Ser
            340                 345                 350

ggg ccc gag gag aag cgt cgc cgt ggc aaa ttg gca ctg cag gag aag      1104
Gly Pro Glu Glu Lys Arg Arg Arg Gly Lys Leu Ala Leu Gln Glu Lys
        355                 360                 365

ccc tcc aca ggt act ctg gaa aaa ctg gtc tct gag gcc aag gcc cag      1152
Pro Ser Thr Gly Thr Leu Glu Lys Leu Val Ser Glu Ala Lys Ala Gln
    370                 375                 380

ctc cga gac att cag gac ttc tgg atc agc ctc cca ggg aca ctg tgc      1200
Leu Arg Asp Ile Gln Asp Phe Trp Ile Ser Leu Pro Gly Thr Leu Cys
385                 390                 395                 400

agt gag aag atg gcc atg agt cct gcc agt gat gac cgc tgc tgg aat      1248
Ser Glu Lys Met Ala Met Ser Pro Ala Ser Asp Asp Arg Cys Trp Asn
                405                 410                 415

gga att tcc aag ggc cgg tac cta cca gag gtg atg ggt gac ggg ctg      1296
Gly Ile Ser Lys Gly Arg Tyr Leu Pro Glu Val Met Gly Asp Gly Leu
            420                 425                 430

gcc aac cag atc aac aac cct gag gtg gaa gtg gac atc acc aag cca      1344
Ala Asn Gln Ile Asn Asn Pro Glu Val Glu Val Asp Ile Thr Lys Pro
        435                 440                 445

gac atg acc atc cgc cag cag att atg cag ctc aag atc atg acc aac      1392
Asp Met Thr Ile Arg Gln Gln Ile Met Gln Leu Lys Ile Met Thr Asn
    450                 455                 460

cgt tta cgt ggc gcc tat ggc ggc aac gac gtg gac ttc cag gat gct      1440
Arg Leu Arg Gly Ala Tyr Gly Gly Asn Asp Val Asp Phe Gln Asp Ala
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465 470 475 480

agt gat gac ggc agt ggc tcc ggc agc ggt ggc gga tgc cca gat gac 1488
Ser Asp Asp Gly Ser Gly Ser Gly Ser Gly Gly Gly Cys Pro Asp Asp
485 490 495

acc tgt ggc cgg agg gtc agc aag aag agt tcc agc tcc cgg acc ccc 1536
Thr Cys Gly Arg Arg Val Ser Lys Lys Ser Ser Ser Ser Arg Thr Pro
500 505 510

ttg acc cat gcc ctc ccc ggc ctg tca gaa cag gag gga cag aag acc 1584
Leu Thr His Ala Leu Pro Gly Leu Ser Glu Gln Glu Gly Gln Lys Thr
515 520 525

tca gct gcc acc tgc cca gag ccc cac agc ttc ttc ctg ctc ttc ctc 1632
Ser Ala Ala Thr Cys Pro Glu Pro His Ser Phe Phe Leu Leu Phe Leu
530 535 540

gtc acc ttg gtc ctt gcg gca gcc agg ccc agg tgg cgg taa 1674
Val Thr Leu Val Leu Ala Ala Ala Arg Pro Arg Trp Arg
545 550 555

<210> 9
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<212> PRT
<213> Mus musculus

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1 5 10 15

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20 25 30

Ser Glu Val Arg Gln Ile Tyr Gly Ala Lys Gly Phe Ser Leu Ser Asp
35 40 45

Val Pro Gln Ala Glu Ile Ser Gly Glu His Leu Arg Ile Cys Pro Gln
50 55 60

Gly Tyr Thr Cys Cys Thr Ser Glu Met Glu Glu Asn Leu Ala Asn His

65 70 75 80

Ser Arg Met Glu Leu Glu Ser Ala Leu His Asp Ser Ser Arg Ala Leu
85 90 95

Gln Ala Thr Leu Ala Thr Gln Leu His Gly Ile Asp Asp His Phe Gln
100 105 110

Arg Leu Leu Asn Asp Ser Glu Arg Thr Leu Gln Glu Ala Phe Pro Gly
115 120 125

Ala Phe Gly Asp Leu Tyr Thr Gln Asn Thr Arg Ala Phe Arg Asp Leu
130 135 140

Tyr Val Glu Leu Arg Leu Tyr Tyr Arg Gly Ala Asn Leu His Leu Glu
145 150 155 160

Glu Thr Leu Ala Glu Phe Trp Ala Arg Leu Leu Glu Arg Leu Phe Lys
165 170 175

Gln Leu His Pro Gln Leu Leu Pro Asp Asp Tyr Leu Asp Cys Leu Gly
180 185 190

Lys Gln Ala Glu Ala Leu Arg Pro Phe Gly Asp Ala Pro Arg Glu Leu
195 200 205

Arg Leu Arg Ala Thr Arg Ala Phe Val Ala Ala Arg Ser Phe Val Gln
210 215 220

Gly Leu Gly Val Ala Ser Asp Val Val Arg Lys Val Ala Gln Val Pro
225 230 235 240

Leu Ala Pro Glu Cys Ser Arg Ala Ile Met Lys Leu Val Tyr Cys Ala
245 250 255

His Cys Arg Gly Val Pro Gly Ala Arg Pro Cys Pro Asp Tyr Cys Arg
260 265 270

Asn Val Leu Lys Gly Cys Leu Ala Asn Gln Ala Asp Leu Asp Ala Glu
275 280 285

Trp Arg Asn Leu Leu Asp Ser Met Val Leu Ile Thr Asp Lys Phe Trp
290 295 300

Gly Pro Ser Gly Ala Glu Ser Val Ile Gly Gly Val His Val Trp Leu
305 310 315 320

Ala Glu Ala Ile Asn Ala Leu Gln Asp Asn Lys Asp Thr Leu Thr Ala
325 330 335

Lys Val Ile Gln Ala Cys Gly Asn Pro Lys Val Asn Pro His Gly Ser
340 345 350

Gly Pro Glu Glu Lys Arg Arg Arg Gly Lys Leu Ala Leu Gln Glu Lys
355 360 365

Pro Ser Thr Gly Thr Leu Glu Lys Leu Val Ser Glu Ala Lys Ala Gln
370 375 380

Leu Arg Asp Ile Gln Asp Phe Trp Ile Ser Leu Pro Gly Thr Leu Cys
385 390 395 400

Ser Glu Lys Met Ala Met Ser Pro Ala Ser Asp Asp Arg Cys Trp Asn
405 410 415

Gly Ile Ser Lys Gly Arg Tyr Leu Pro Glu Val Met Gly Asp Gly Leu
420 425 430

Ala Asn Gln Ile Asn Asn Pro Glu Val Glu Val Asp Ile Thr Lys Pro

435 440 445

Asp Met Thr Ile Arg Gln Gln Ile Met Gln Leu Lys Ile Met Thr Asn
450 455 460

Arg Leu Arg Gly Ala Tyr Gly Gly Asn Asp Val Asp Phe Gln Asp Ala
465 470 475 480

Ser Asp Asp Gly Ser Gly Ser Gly Ser Gly Gly Gly Cys Pro Asp Asp
485 490 495

Thr Cys Gly Arg Arg Val Ser Lys Lys Ser Ser Ser Ser Arg Thr Pro
500 505 510

Leu Thr His Ala Leu Pro Gly Leu Ser Glu Gln Glu Gly Gln Lys Thr
515 520 525

Ser Ala Ala Thr Cys Pro Glu Pro His Ser Phe Phe Leu Leu Phe Leu
530 535 540

Val Thr Leu Val Leu Ala Ala Ala Arg Pro Arg Trp Arg
545 550 555

<210> 10
<211> 1677
<212> DNA
<213> Homo sapiens

<220>
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<222> (1)..(1674)

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Met Glu Leu Arg Ala Arg Gly Trp Trp Leu Leu Cys Ala Ala Ala Ala
1 5 10 15

ctg gtc gcc tgc gcc cgc ggg gac ccg gcc agc aag agc cgg agc tgc 96

Leu Val Ala Cys Ala Arg Gly Asp Pro Ala Ser Lys Ser Arg Ser Cys
20 25 30

ggc gag gtc cgc cag atc tac gga gcc aag ggc ttc agc ctg agc gac 144
Gly Glu Val Arg Gln Ile Tyr Gly Ala Lys Gly Phe Ser Leu Ser Asp
35 40 45

gtg ccc cag gcg gag atc tcg ggt gag cac ctg cgg atc tgt ccc cag 192
Val Pro Gln Ala Glu Ile Ser Gly Glu His Leu Arg Ile Cys Pro Gln
50 55 60

ggc tac acc tgc tgc acc agc gag atg gag gag aac ctg gcc aac cgc 240
Gly Tyr Thr Cys Cys Thr Ser Glu Met Glu Glu Asn Leu Ala Asn Arg
65 70 75 80

agc cat gcc gag ctg gag acc gcg ctc cgg gac agc agc cgc gtc ctg 288
Ser His Ala Glu Leu Glu Thr Ala Leu Arg Asp Ser Ser Arg Val Leu
85 90 95

cag gcc atg ctt gcc acc cag ctg cgc agc ttc gat gac cac ttc cag 336
Gln Ala Met Leu Ala Thr Gln Leu Arg Ser Phe Asp Asp His Phe Gln
100 105 110

cac ctg ctg aac gac tcg gag cgg acg ctg cag gcc acc ttc ccc ggc 384
His Leu Leu Asn Asp Ser Glu Arg Thr Leu Gln Ala Thr Phe Pro Gly
115 120 125

gcc ttc gga gag ctg tac acg cag aac gcg agg gcc ttc cgg gac ctg 432
Ala Phe Gly Glu Leu Tyr Thr Gln Asn Ala Arg Ala Phe Arg Asp Leu
130 135 140

tac tca gag ctg cgc ctg tac tac cgc ggt gcc aac ctg cac ctg gag 480
Tyr Ser Glu Leu Arg Leu Tyr Tyr Arg Gly Ala Asn Leu His Leu Glu
145 150 155 160

gag acg ctg gcc gag ttc tgg gcc cgc ctg ctc gag cgc ctc ttc aag 528
Glu Thr Leu Ala Glu Phe Trp Ala Arg Leu Leu Glu Arg Leu Phe Lys
165 170 175

cag ctg cac ccc cag ctg ctg ctg cct gat gac tac ctg gac tgc ctg 576
Gln Leu His Pro Gln Leu Leu Leu Pro Asp Asp Tyr Leu Asp Cys Leu
180 185 190

ggc aag cag gcc gag gcg ctg cgg ccc ttc ggg gag gcc ccg aga gag 624
Gly Lys Gln Ala Glu Ala Leu Arg Pro Phe Gly Glu Ala Pro Arg Glu
195 200 205

ctg cgc ctg cgg gcc acc cgt gcc ttc gtg gct gct cgc tcc ttt gtg 672
Leu Arg Leu Arg Ala Thr Arg Ala Phe Val Ala Ala Arg Ser Phe Val
210 215 220

cag ggc ctg ggc gtg gcc agc gac gtg gtc cgg aaa gtg gct cag gtc 720
Gln Gly Leu Gly Val Ala Ser Asp Val Val Arg Lys Val Ala Gln Val
225 230 235 240

ccc ctg ggc ccg gag tgc tcg aga gct gtc atg aag ctg gtc tac tgt 768
Pro Leu Gly Pro Glu Cys Ser Arg Ala Val Met Lys Leu Val Tyr Cys
245 250 255

gct cac tgc ctg gga gtc ccc ggc gcc agg ccc tgc cct gac tat tgc 816
Ala His Cys Leu Gly Val Pro Gly Ala Arg Pro Cys Pro Asp Tyr Cys
260 265 270

cga aat gtg ctc aag ggc tgc ctt gcc aac cag gcc gac ctg gac gcc 864
Arg Asn Val Leu Lys Gly Cys Leu Ala Asn Gln Ala Asp Leu Asp Ala
275 280 285

gag tgg agg aac ctc ctg gac tcc atg gtg ctc atc acc gac aag ttc 912
Glu Trp Arg Asn Leu Leu Asp Ser Met Val Leu Ile Thr Asp Lys Phe
290 295 300

tgg ggt aca tcg ggt gtg gag agt gtc atc ggc agc gtg cac acg tgg 960
Trp Gly Thr Ser Gly Val Glu Ser Val Ile Gly Ser Val His Thr Trp
305 310 315 320

ctg gcg gag gcc atc aac gcc ctc cag gac aac agg gac acg ctc acg 1008
Leu Ala Glu Ala Ile Asn Ala Leu Gln Asp Asn Arg Asp Thr Leu Thr
325 330 335

gcc aag gtc atc cag ggc tgc ggg aac ccc aag gtc aac ccc cag ggc 1056
Ala Lys Val Ile Gln Gly Cys Gly Asn Pro Lys Val Asn Pro Gln Gly
340 345 350

cct ggg cct gag gag aag cgg cgc cgg ggc aag ctg gcc ccg cgg gag 1104
Pro Gly Pro Glu Glu Lys Arg Arg Arg Gly Lys Leu Ala Pro Arg Glu
355 360 365

agg cca cct tca ggc acg ctg gag aag ctg gtc tct gaa gcc aag gcc 1152
Arg Pro Pro Ser Gly Thr Leu Glu Lys Leu Val Ser Glu Ala Lys Ala
370 375 380

cag ctc cgc gac gtc cag gac ttc tgg atc agc ctc cca ggg aca ctg 1200

Gln Leu Arg Asp Val Gln Asp Phe Trp Ile Ser Leu Pro Gly Thr Leu
385 390 395 400

tgc agt gag aag atg gcc ctg agc act gcc agt gat gac cgc tgc tgg 1248
Cys Ser Glu Lys Met Ala Leu Ser Thr Ala Ser Asp Asp Arg Cys Trp
405 410 415

aac ggg atg gcc aga ggc cgg tac ctc ccc gag gtc atg ggt gac ggc 1296
Asn Gly Met Ala Arg Gly Arg Tyr Leu Pro Glu Val Met Gly Asp Gly
420 425 430

ctg gcc aac cag atc aac aac ccc gag gtg gag gtg gac atc acc aag 1344
Leu Ala Asn Gln Ile Asn Asn Pro Glu Val Glu Val Asp Ile Thr Lys
435 440 445

ccg gac atg acc atc cgg cag cag atc atg cag ctg aag atc atg acc 1392
Pro Asp Met Thr Ile Arg Gln Gln Ile Met Gln Leu Lys Ile Met Thr
450 455 460

aac cgg ctg cgc agc gcc tac aac ggc aac gac gtg gac ttc cag gac 1440
Asn Arg Leu Arg Ser Ala Tyr Asn Gly Asn Asp Val Asp Phe Gln Asp
465 470 475 480

gcc agt gac gac ggc agc ggc tcg ggc agc ggt gat ggc tgt ctg gat 1488
Ala Ser Asp Asp Gly Ser Gly Ser Gly Ser Gly Asp Gly Cys Leu Asp
485 490 495

gac ctc tgc ggc cgg aag gtc agc agg aag agc tcc agc tcc cgg acg 1536
Asp Leu Cys Gly Arg Lys Val Ser Arg Lys Ser Ser Ser Ser Arg Thr
500 505 510

ccc ttg acc cat gcc ctc cca ggc ctg tca gag cag gaa gga cag aag 1584
Pro Leu Thr His Ala Leu Pro Gly Leu Ser Glu Gln Glu Gly Gln Lys
515 520 525

acc tcg gct gcc agc tgc ccc cag ccc ccg acc ttc ctc ctg ccc ctc 1632
Thr Ser Ala Ala Ser Cys Pro Gln Pro Pro Thr Phe Leu Leu Pro Leu
530 535 540

ctc ctc ttc ctg gcc ctt aca gta gcc agg ccc cgg tgg cgg taa 1677
Leu Leu Phe Leu Ala Leu Thr Val Ala Arg Pro Arg Trp Arg
545 550 555

<210> 11
<211> 558

<212> PRT
<213> Homo sapiens

<400> 11
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1 5 10 15

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Gly Glu Val Arg Gln Ile Tyr Gly Ala Lys Gly Phe Ser Leu Ser Asp
35 40 45

Val Pro Gln Ala Glu Ile Ser Gly Glu His Leu Arg Ile Cys Pro Gln
50 55 60

Gly Tyr Thr Cys Cys Thr Ser Glu Met Glu Glu Asn Leu Ala Asn Arg
65 70 75 80

Ser His Ala Glu Leu Glu Thr Ala Leu Arg Asp Ser Ser Arg Val Leu
85 90 95

Gln Ala Met Leu Ala Thr Gln Leu Arg Ser Phe Asp Asp His Phe Gln
100 105 110

His Leu Leu Asn Asp Ser Glu Arg Thr Leu Gln Ala Thr Phe Pro Gly
115 120 125

Ala Phe Gly Glu Leu Tyr Thr Gln Asn Ala Arg Ala Phe Arg Asp Leu
130 135 140

Tyr Ser Glu Leu Arg Leu Tyr Tyr Arg Gly Ala Asn Leu His Leu Glu
145 150 155 160

Glu Thr Leu Ala Glu Phe Trp Ala Arg Leu Leu Glu Arg Leu Phe Lys
165 170 175

Gln Leu His Pro Gln Leu Leu Leu Pro Asp Asp Tyr Leu Asp Cys Leu
180 185 190

Gly Lys Gln Ala Glu Ala Leu Arg Pro Phe Gly Glu Ala Pro Arg Glu
195 200 205

Leu Arg Leu Arg Ala Thr Arg Ala Phe Val Ala Ala Arg Ser Phe Val
210 215 220

Gln Gly Leu Gly Val Ala Ser Asp Val Val Arg Lys Val Ala Gln Val
225 230 235 240

Pro Leu Gly Pro Glu Cys Ser Arg Ala Val Met Lys Leu Val Tyr Cys
245 250 255

Ala His Cys Leu Gly Val Pro Gly Ala Arg Pro Cys Pro Asp Tyr Cys
260 265 270

Arg Asn Val Leu Lys Gly Cys Leu Ala Asn Gln Ala Asp Leu Asp Ala
275 280 285

Glu Trp Arg Asn Leu Leu Asp Ser Met Val Leu Ile Thr Asp Lys Phe
290 295 300

Trp Gly Thr Ser Gly Val Glu Ser Val Ile Gly Ser Val His Thr Trp
305 310 315 320

Leu Ala Glu Ala Ile Asn Ala Leu Gln Asp Asn Arg Asp Thr Leu Thr
325 330 335

Ala Lys Val Ile Gln Gly Cys Gly Asn Pro Lys Val Asn Pro Gln Gly
340 345 350

Pro Gly Pro Glu Glu Lys Arg Arg Arg Gly Lys Leu Ala Pro Arg Glu
        355   360   365

Arg Pro Pro Ser Gly Thr Leu Glu Lys Leu Val Ser Glu Ala Lys Ala
    370   375   380

Gln Leu Arg Asp Val Gln Asp Phe Trp Ile Ser Leu Pro Gly Thr Leu
385   390   395   400

Cys Ser Glu Lys Met Ala Leu Ser Thr Ala Ser Asp Asp Arg Cys Trp
                405   410   415

Asn Gly Met Ala Arg Gly Arg Tyr Leu Pro Glu Val Met Gly Asp Gly
            420   425   430

Leu Ala Asn Gln Ile Asn Asn Pro Glu Val Glu Val Asp Ile Thr Lys
        435   440   445

Pro Asp Met Thr Ile Arg Gln Gln Ile Met Gln Leu Lys Ile Met Thr
    450   455   460

Asn Arg Leu Arg Ser Ala Tyr Asn Gly Asn Asp Val Asp Phe Gln Asp
465   470   475   480

Ala Ser Asp Asp Gly Ser Gly Ser Gly Ser Gly Asp Gly Cys Leu Asp
                485   490   495

Asp Leu Cys Gly Arg Lys Val Ser Arg Lys Ser Ser Ser Ser Arg Thr
            500   505   510

Pro Leu Thr His Ala Leu Pro Gly Leu Ser Glu Gln Glu Gly Gln Lys
        515   520   525

Thr Ser Ala Ala Ser Cys Pro Gln Pro Pro Thr Phe Leu Leu Pro Leu
    530   535   540

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Leu Leu Phe Leu Ala Leu Thr Val Ala Arg Pro Arg Trp Arg
545                 550                 555


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<212>  DNA
<213>  Mus musculus

<220>
<221>  CDS
<222>  (1)..(366)

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Met Lys Pro Phe His Thr Ala Leu Ser Phe Leu Ile Leu Thr Thr Ala
1               5                   10                  15

ctt gga atc tgg gcc cag atc aca cat gca aca gag aca aaa gaa gtc        96
Leu Gly Ile Trp Ala Gln Ile Thr His Ala Thr Glu Thr Lys Glu Val
            20                  25                  30

cag agc agt ctg aag gca cag caa ggg ctt gaa att gaa atg ttt cac       144
Gln Ser Ser Leu Lys Ala Gln Gln Gly Leu Glu Ile Glu Met Phe His
        35                  40                  45

atg ggc ttt caa gac tct tca gat tgc tgc ctg tcc tat aac tca cgg       192
Met Gly Phe Gln Asp Ser Ser Asp Cys Cys Leu Ser Tyr Asn Ser Arg
    50                  55                  60

att cag tgt tca aga ttt ata ggt tat ttt ccc acc agt ggt ggg tgt       240
Ile Gln Cys Ser Arg Phe Ile Gly Tyr Phe Pro Thr Ser Gly Gly Cys
65                  70                  75                  80

acc agg ccg ggc atc atc ttt atc agc aag agg ggg ttc cag gtc tgt       288
Thr Arg Pro Gly Ile Ile Phe Ile Ser Lys Arg Gly Phe Gln Val Cys
                85                  90                  95

gcc aac ccc agt gat cgg aga gtt cag aga tgc att gaa aga ttg gag       336
Ala Asn Pro Ser Asp Arg Arg Val Gln Arg Cys Ile Glu Arg Leu Glu
            100                 105                 110

caa aac tca caa cca cgg acc tac aaa caa taa                           369
Gln Asn Ser Gln Pro Arg Thr Tyr Lys Gln
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115 120

<210> 13
<211> 122
<212> PRT
<213> Mus musculus

<400> 13
Met Lys Pro Phe His Thr Ala Leu Ser Phe Leu Ile Leu Thr Thr Ala
1 5 10 15

Leu Gly Ile Trp Ala Gln Ile Thr His Ala Thr Glu Thr Lys Glu Val
20 25 30

Gln Ser Ser Leu Lys Ala Gln Gln Gly Leu Glu Ile Glu Met Phe His
35 40 45

Met Gly Phe Gln Asp Ser Ser Asp Cys Cys Leu Ser Tyr Asn Ser Arg
50 55 60

Ile Gln Cys Ser Arg Phe Ile Gly Tyr Phe Pro Thr Ser Gly Gly Cys
65 70 75 80

Thr Arg Pro Gly Ile Ile Phe Ile Ser Lys Arg Gly Phe Gln Val Cys
85 90 95

Ala Asn Pro Ser Asp Arg Arg Val Gln Arg Cys Ile Glu Arg Leu Glu
100 105 110

Gln Asn Ser Gln Pro Arg Thr Tyr Lys Gln
115 120

<210> 14
<211> 1223
<212> DNA
<213> Mus musculus

<220>
<221> CDS
<222> (84)..(1121)

<400> 14

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gtgacccgga agggagcccc gtggtagagg tgaccggagc tgagcatttc agatctgctt      60

agtaaaccgg tgtatcgccc acc atg ttg gct gca agg ctt gtg tgt ctc cgg     113
                          Met Leu Ala Ala Arg Leu Val Cys Leu Arg
                          1               5                   10

aca cta cct tcc agg gtt ttc cag ccc act ttc atc acc aag gcc tct       161
Thr Leu Pro Ser Arg Val Phe Gln Pro Thr Phe Ile Thr Lys Ala Ser
                15                  20                  25

cca ctt gtg aag aat tcc atc aca aag aac caa tgg ctc gta aca ccc       209
Pro Leu Val Lys Asn Ser Ile Thr Lys Asn Gln Trp Leu Val Thr Pro
            30                  35                  40

agc agg gaa tat gct acc aag aca aga att agg act cac cgt ggg aaa       257
Ser Arg Glu Tyr Ala Thr Lys Thr Arg Ile Arg Thr His Arg Gly Lys
        45                  50                  55

act gga caa gaa ctg aaa gag gca gcc ttg gaa cca tca atg gaa aaa       305
Thr Gly Gln Glu Leu Lys Glu Ala Ala Leu Glu Pro Ser Met Glu Lys
    60                  65                  70

atc ttt aaa atc gat caa atg gga agg tgg ttt gtt gct gga gga gca       353
Ile Phe Lys Ile Asp Gln Met Gly Arg Trp Phe Val Ala Gly Gly Ala
75                  80                  85                  90

gct gtt ggt ctt gga gcg ctc tgc tac tat ggc ttg gga atg tct aat       401
Ala Val Gly Leu Gly Ala Leu Cys Tyr Tyr Gly Leu Gly Met Ser Asn
                95                  100                 105

gag att gga gct atc gaa aag gct gta att tgg cct cag tat gta aag       449
Glu Ile Gly Ala Ile Glu Lys Ala Val Ile Trp Pro Gln Tyr Val Lys
            110                 115                 120

gat aga att cat tct act tac atg tac tta gca gga agg tat tgt tta       497
Asp Arg Ile His Ser Thr Tyr Met Tyr Leu Ala Gly Arg Tyr Cys Leu
        125                 130                 135

aca gct ttg tct gcc ttg gca gta gcc aga aca cct gct ctc atg aac       545
Thr Ala Leu Ser Ala Leu Ala Val Ala Arg Thr Pro Ala Leu Met Asn
    140                 145                 150
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ttc atg atg aca ggc tct tgg gtg aca att ggt gcg acc ttt gca gcc 593
Phe Met Met Thr Gly Ser Trp Val Thr Ile Gly Ala Thr Phe Ala Ala
155 160 165 170

atg att gga gct gga atg ctt gta cac tca ata tca tat gag cag agc 641
Met Ile Gly Ala Gly Met Leu Val His Ser Ile Ser Tyr Glu Gln Ser
175 180 185

cca ggc cca aag cat ctg gct tgg atg ctg cat tct ggt gtg atg ggt 689
Pro Gly Pro Lys His Leu Ala Trp Met Leu His Ser Gly Val Met Gly
190 195 200

gca gtt gtg gct cct ctg acg atc tta ggg ggg cct ctt ctc ctg aga 737
Ala Val Val Ala Pro Leu Thr Ile Leu Gly Gly Pro Leu Leu Leu Arg
205 210 215

gcc gca tgg tac acc gct ggt att gtg gga ggc ctc tct act gtg gcc 785
Ala Ala Trp Tyr Thr Ala Gly Ile Val Gly Gly Leu Ser Thr Val Ala
220 225 230

atg tgt gcg cct agt gag aag ttt ctg aac atg gga gca ccc ctg gga 833
Met Cys Ala Pro Ser Glu Lys Phe Leu Asn Met Gly Ala Pro Leu Gly
235 240 245 250

gtg ggc ctg ggt ctt gtc ttt gcg tct tct ctg ggg tct atg ttt ctt 881
Val Gly Leu Gly Leu Val Phe Ala Ser Ser Leu Gly Ser Met Phe Leu
255 260 265

ccc cct acc tct gtg gct ggt gcc act ctg tac tca gtg gca atg tat 929
Pro Pro Thr Ser Val Ala Gly Ala Thr Leu Tyr Ser Val Ala Met Tyr
270 275 280

ggt gga tta gtt ctt ttc agc atg ttc ctt ctg tat gat act cag aaa 977
Gly Gly Leu Val Leu Phe Ser Met Phe Leu Leu Tyr Asp Thr Gln Lys
285 290 295

gta atc aaa cgt gca gaa ata aca ccc atg tat gga gct caa aag tat 1025
Val Ile Lys Arg Ala Glu Ile Thr Pro Met Tyr Gly Ala Gln Lys Tyr
300 305 310

gat ccc atc aat tcg atg ttg aca atc tac atg gat aca tta aat ata 1073
Asp Pro Ile Asn Ser Met Leu Thr Ile Tyr Met Asp Thr Leu Asn Ile
315 320 325 330

ttt atg cga gtt gca act atg cta gca act gga agc aac aga aag aaa 1121

Phe Met Arg Val Ala Thr Met Leu Ala Thr Gly Ser Asn Arg Lys Lys
335 340 345

tgaagtaacc gcttgtgatg tctccgctca ctgatgtctt gcttgtttaa taggagcaga 1181

tagtcattac agtttgcatc agcagaattc ccgcgcggcc gc 1223

<210> 15
<211> 346
<212> PRT
<213> Mus musculus

<400> 15
Met Leu Ala Ala Arg Leu Val Cys Leu Arg Thr Leu Pro Ser Arg Val
1 5 10 15

Phe Gln Pro Thr Phe Ile Thr Lys Ala Ser Pro Leu Val Lys Asn Ser
20 25 30

Ile Thr Lys Asn Gln Trp Leu Val Thr Pro Ser Arg Glu Tyr Ala Thr
35 40 45

Lys Thr Arg Ile Arg Thr His Arg Gly Lys Thr Gly Gln Glu Leu Lys
50 55 60

Glu Ala Ala Leu Glu Pro Ser Met Glu Lys Ile Phe Lys Ile Asp Gln
65 70 75 80

Met Gly Arg Trp Phe Val Ala Gly Gly Ala Ala Val Gly Leu Gly Ala
85 90 95

Leu Cys Tyr Tyr Gly Leu Gly Met Ser Asn Glu Ile Gly Ala Ile Glu
100 105 110

Lys Ala Val Ile Trp Pro Gln Tyr Val Lys Asp Arg Ile His Ser Thr
115 120 125

Tyr Met Tyr Leu Ala Gly Arg Tyr Cys Leu Thr Ala Leu Ser Ala Leu
    130   135   140

Ala Val Ala Arg Thr Pro Ala Leu Met Asn Phe Met Met Thr Gly Ser
145   150   155   160

Trp Val Thr Ile Gly Ala Thr Phe Ala Ala Met Ile Gly Ala Gly Met
                165   170   175

Leu Val His Ser Ile Ser Tyr Glu Gln Ser Pro Gly Pro Lys His Leu
            180   185   190

Ala Trp Met Leu His Ser Gly Val Met Gly Ala Val Val Ala Pro Leu
        195   200   205

Thr Ile Leu Gly Gly Pro Leu Leu Leu Arg Ala Ala Trp Tyr Thr Ala
    210   215   220

Gly Ile Val Gly Gly Leu Ser Thr Val Ala Met Cys Ala Pro Ser Glu
225   230   235   240

Lys Phe Leu Asn Met Gly Ala Pro Leu Gly Val Gly Leu Gly Leu Val
                245   250   255

Phe Ala Ser Ser Leu Gly Ser Met Phe Leu Pro Pro Thr Ser Val Ala
            260   265   270

Gly Ala Thr Leu Tyr Ser Val Ala Met Tyr Gly Gly Leu Val Leu Phe
        275   280   285

Ser Met Phe Leu Leu Tyr Asp Thr Gln Lys Val Ile Lys Arg Ala Glu
    290   295   300

Ile Thr Pro Met Tyr Gly Ala Gln Lys Tyr Asp Pro Ile Asn Ser Met
305   310   315   320

Leu Thr Ile Tyr Met Asp Thr Leu Asn Ile Phe Met Arg Val Ala Thr
325 330 335

Met Leu Ala Thr Gly Ser Asn Arg Lys Lys
340 345

<210> 16
<211> 1038
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (1)..(1035)

<400> 16
atg ttg gct gca agg ctg gtg tgt ctc cgg aca cta cct tct agg gtt 48
Met Leu Ala Ala Arg Leu Val Cys Leu Arg Thr Leu Pro Ser Arg Val
1 5 10 15

ttc cac cca gct ttc acc aag gcc tcc cct gtt gtg aag aat tcc atc 96
Phe His Pro Ala Phe Thr Lys Ala Ser Pro Val Val Lys Asn Ser Ile
20 25 30

acg aag aat caa tgg ctg tta aca cct agc agg gaa tat gcc acc aaa 144
Thr Lys Asn Gln Trp Leu Leu Thr Pro Ser Arg Glu Tyr Ala Thr Lys
35 40 45

aca aga att ggg atc cgg cgt ggg aga act ggc caa gaa ctc aaa gag 192
Thr Arg Ile Gly Ile Arg Arg Gly Arg Thr Gly Gln Glu Leu Lys Glu
50 55 60

gca gca ttg gaa cca tcg atg gaa aaa ata ttt aaa att gat cag atg 240
Ala Ala Leu Glu Pro Ser Met Glu Lys Ile Phe Lys Ile Asp Gln Met
65 70 75 80

gga aga tgg ttt gtt gct gga ggg gct gct gtt ggt ctt gga gca ttg 288
Gly Arg Trp Phe Val Ala Gly Gly Ala Ala Val Gly Leu Gly Ala Leu
85 90 95

tgc tac tat ggc ttg gga ctg tct aat gag att gga gct att gaa aag 336
Cys Tyr Tyr Gly Leu Gly Leu Ser Asn Glu Ile Gly Ala Ile Glu Lys

100 105 110

gct gta att tgg cct cag tat gtc aag gat aga att cat tcc acc tat 384
Ala Val Ile Trp Pro Gln Tyr Val Lys Asp Arg Ile His Ser Thr Tyr
115 120 125

atg tac tta gca ggg agt att ggt tta aca gct ttg tct gcc ata gca 432
Met Tyr Leu Ala Gly Ser Ile Gly Leu Thr Ala Leu Ser Ala Ile Ala
130 135 140

atc agc aga acg cct gtt ctc atg aac ttc atg atg aga ggc tct tgg 480
Ile Ser Arg Thr Pro Val Leu Met Asn Phe Met Met Arg Gly Ser Trp
145 150 155 160

gtg aca att ggt gtg acc ttt gca gcc atg gtt gga gct gga atg ctg 528
Val Thr Ile Gly Val Thr Phe Ala Ala Met Val Gly Ala Gly Met Leu
165 170 175

gta cga tca ata cca tat gac cag agc cca ggc cca aag cat ctt gct 576
Val Arg Ser Ile Pro Tyr Asp Gln Ser Pro Gly Pro Lys His Leu Ala
180 185 190

tgg ttg cta cat tct ggt gtg atg ggt gca gtg gtg gct cct ctg aca 624
Trp Leu Leu His Ser Gly Val Met Gly Ala Val Val Ala Pro Leu Thr
195 200 205

ata tta ggg ggt cct ctt ctc atc aga gct gca tgg tac aca gct ggc 672
Ile Leu Gly Gly Pro Leu Leu Ile Arg Ala Ala Trp Tyr Thr Ala Gly
210 215 220

att gtg gga ggc ctc tcc act gtg gcc atg tgt gcg ccc agt gaa aag 720
Ile Val Gly Gly Leu Ser Thr Val Ala Met Cys Ala Pro Ser Glu Lys
225 230 235 240

ttt ctg aac atg ggt gca ccc ctg gga gtg ggc ctg ggt ctc gtc ttt 768
Phe Leu Asn Met Gly Ala Pro Leu Gly Val Gly Leu Gly Leu Val Phe
245 250 255

gtg tcc tca ttg gga tct atg ttt ctt cca cct acc acc gtg gct ggt 816
Val Ser Ser Leu Gly Ser Met Phe Leu Pro Pro Thr Thr Val Ala Gly
260 265 270

gcc act ctt tac tca gtg gca atg tac ggt gga tta gtt ctt ttc agc 864
Ala Thr Leu Tyr Ser Val Ala Met Tyr Gly Gly Leu Val Leu Phe Ser
275 280 285

atg ttc ctt ctg tat gat acc cag aaa gta atc aag cgt gca gaa gta 912
Met Phe Leu Leu Tyr Asp Thr Gln Lys Val Ile Lys Arg Ala Glu Val
290 295 300

tca cca atg tat gga gtt caa aaa tat gat ccc att aac tcg atg ctg 960
Ser Pro Met Tyr Gly Val Gln Lys Tyr Asp Pro Ile Asn Ser Met Leu
305 310 315 320

agt atc tac atg gat aca tta aat ata ttt atg cga gtt gca act atg 1008
Ser Ile Tyr Met Asp Thr Leu Asn Ile Phe Met Arg Val Ala Thr Met
325 330 335

ctg gca act gga ggc aac aga aag aaa tga 1038
Leu Ala Thr Gly Gly Asn Arg Lys Lys
340 345

<210> 17
<211> 345
<212> PRT
<213> Homo sapiens

<400> 17
Met Leu Ala Ala Arg Leu Val Cys Leu Arg Thr Leu Pro Ser Arg Val
1 5 10 15

Phe His Pro Ala Phe Thr Lys Ala Ser Pro Val Val Lys Asn Ser Ile
20 25 30

Thr Lys Asn Gln Trp Leu Leu Thr Pro Ser Arg Glu Tyr Ala Thr Lys
35 40 45

Thr Arg Ile Gly Ile Arg Arg Gly Arg Thr Gly Gln Glu Leu Lys Glu
50 55 60

Ala Ala Leu Glu Pro Ser Met Glu Lys Ile Phe Lys Ile Asp Gln Met
65 70 75 80

Gly Arg Trp Phe Val Ala Gly Gly Ala Ala Val Gly Leu Gly Ala Leu
85 90 95

Cys Tyr Tyr Gly Leu Gly Leu Ser Asn Glu Ile Gly Ala Ile Glu Lys
            100   105   110

Ala Val Ile Trp Pro Gln Tyr Val Lys Asp Arg Ile His Ser Thr Tyr
        115   120   125

Met Tyr Leu Ala Gly Ser Ile Gly Leu Thr Ala Leu Ser Ala Ile Ala
    130   135   140

Ile Ser Arg Thr Pro Val Leu Met Asn Phe Met Met Arg Gly Ser Trp
145   150   155   160

Val Thr Ile Gly Val Thr Phe Ala Ala Met Val Gly Ala Gly Met Leu
                165   170   175

Val Arg Ser Ile Pro Tyr Asp Gln Ser Pro Gly Pro Lys His Leu Ala
            180   185   190

Trp Leu Leu His Ser Gly Val Met Gly Ala Val Val Ala Pro Leu Thr
        195   200   205

Ile Leu Gly Gly Pro Leu Leu Ile Arg Ala Ala Trp Tyr Thr Ala Gly
    210   215   220

Ile Val Gly Gly Leu Ser Thr Val Ala Met Cys Ala Pro Ser Glu Lys
225   230   235   240

Phe Leu Asn Met Gly Ala Pro Leu Gly Val Gly Leu Gly Leu Val Phe
                245   250   255

Val Ser Ser Leu Gly Ser Met Phe Leu Pro Pro Thr Thr Val Ala Gly
            260   265   270

Ala Thr Leu Tyr Ser Val Ala Met Tyr Gly Gly Leu Val Leu Phe Ser

275 280 285

Met Phe Leu Leu Tyr Asp Thr Gln Lys Val Ile Lys Arg Ala Glu Val
290 295 300

Ser Pro Met Tyr Gly Val Gln Lys Tyr Asp Pro Ile Asn Ser Met Leu
305 310 315 320

Ser Ile Tyr Met Asp Thr Leu Asn Ile Phe Met Arg Val Ala Thr Met
325 330 335

Leu Ala Thr Gly Gly Asn Arg Lys Lys
340 345

<210> 18
<211> 447
<212> DNA
<213> Mus musculus

<220>
<221> CDS
<222> (1)..(444)

<400> 18
atg agc acc tcg tct gcg cgg cct gca gtc ctg gcc ctt gcc ggg ctg 48
Met Ser Thr Ser Ser Ala Arg Pro Ala Val Leu Ala Leu Ala Gly Leu
1 5 10 15

gct ctg ctc ctt ctg ctg tgc ctg ggt cca gat ggc ata agt gga aac 96
Ala Leu Leu Leu Leu Leu Cys Leu Gly Pro Asp Gly Ile Ser Gly Asn
20 25 30

aaa ctc aag aag atg ctc cag aaa cga gaa gga cct gtc ccg tca aag 144
Lys Leu Lys Lys Met Leu Gln Lys Arg Glu Gly Pro Val Pro Ser Lys
35 40 45

act aat gta gct gta gcc gag aac aca gca aag gaa ttc cta ggt ggc 192
Thr Asn Val Ala Val Ala Glu Asn Thr Ala Lys Glu Phe Leu Gly Gly
50 55 60

ctg aag cgt gcc aaa cga cag ctg tgg gac cgt acg cgg cct gag gta 240

Leu Lys Arg Ala Lys Arg Gln Leu Trp Asp Arg Thr Arg Pro Glu Val
65 70 75 80

cag cag tgg tac cag cag ttc ctc tac atg ggc ttt gat gag gct aaa 288
Gln Gln Trp Tyr Gln Gln Phe Leu Tyr Met Gly Phe Asp Glu Ala Lys
85 90 95

ttt gaa gat gat gtc aac tat tgg cta aac aga aat cga aac ggc cat 336
Phe Glu Asp Asp Val Asn Tyr Trp Leu Asn Arg Asn Arg Asn Gly His
100 105 110

gac tac tat ggt gac tac tac cag cgt cat tat gat gaa gat gcg gcc 384
Asp Tyr Tyr Gly Asp Tyr Tyr Gln Arg His Tyr Asp Glu Asp Ala Ala
115 120 125

att ggt ccc cac agc cgg gaa agc ttc agg cat gga gcc agt gtg aac 432
Ile Gly Pro His Ser Arg Glu Ser Phe Arg His Gly Ala Ser Val Asn
130 135 140

tat gat gac tat taa 447
Tyr Asp Asp Tyr
145

<210> 19
<211> 148
<212> PRT
<213> Mus musculus

<400> 19
Met Ser Thr Ser Ser Ala Arg Pro Ala Val Leu Ala Leu Ala Gly Leu
1 5 10 15

Ala Leu Leu Leu Leu Leu Cys Leu Gly Pro Asp Gly Ile Ser Gly Asn
20 25 30

Lys Leu Lys Lys Met Leu Gln Lys Arg Glu Gly Pro Val Pro Ser Lys
35 40 45

Thr Asn Val Ala Val Ala Glu Asn Thr Ala Lys Glu Phe Leu Gly Gly
50 55 60

Leu Lys Arg Ala Lys Arg Gln Leu Trp Asp Arg Thr Arg Pro Glu Val
65 70 75 80

Gln Gln Trp Tyr Gln Gln Phe Leu Tyr Met Gly Phe Asp Glu Ala Lys
85 90 95

Phe Glu Asp Asp Val Asn Tyr Trp Leu Asn Arg Asn Arg Asn Gly His
100 105 110

Asp Tyr Tyr Gly Asp Tyr Tyr Gln Arg His Tyr Asp Glu Asp Ala Ala
115 120 125

Ile Gly Pro His Ser Arg Glu Ser Phe Arg His Gly Ala Ser Val Asn
130 135 140

Tyr Asp Asp Tyr
145

<210> 20
<211> 447
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (1)..(444)

<400> 20
atg gct gcc tcc ccc gcg cgg cct gct gtc ctg gcc ctg acc ggg ctg 48
Met Ala Ala Ser Pro Ala Arg Pro Ala Val Leu Ala Leu Thr Gly Leu
1 5 10 15

gcg ctg ctc ctg ctc ctg tgc tgg ggc cca ggt ggc ata agt gga aat 96
Ala Leu Leu Leu Leu Leu Cys Trp Gly Pro Gly Gly Ile Ser Gly Asn
20 25 30

aaa ctc aag ctg atg ctt caa aaa cga gaa gca cct gtt cca act aag 144
Lys Leu Lys Leu Met Leu Gln Lys Arg Glu Ala Pro Val Pro Thr Lys
35 40 45

act aaa gtg gcc gtt gat gag aat aaa gcc aaa gaa ttc ctt ggc agc 192
Thr Lys Val Ala Val Asp Glu Asn Lys Ala Lys Glu Phe Leu Gly Ser
50 55 60

ctg aag cgc cag aag cgg cag ctg tgg gac cgg act cgg ccc gag gtg 240
Leu Lys Arg Gln Lys Arg Gln Leu Trp Asp Arg Thr Arg Pro Glu Val
65 70 75 80

cag cag tgg tac cag cag ttt ctc tac atg ggc ttt gac gaa gcg aaa 288
Gln Gln Trp Tyr Gln Gln Phe Leu Tyr Met Gly Phe Asp Glu Ala Lys
85 90 95

ttt gaa gat gac atc acc tat tgg ctt aac aga gat cga aat gga cat 336
Phe Glu Asp Asp Ile Thr Tyr Trp Leu Asn Arg Asp Arg Asn Gly His
100 105 110

gaa tac tat ggc gat tac tac caa cgt cac tat gat gaa gac tct gca 384
Glu Tyr Tyr Gly Asp Tyr Tyr Gln Arg His Tyr Asp Glu Asp Ser Ala
115 120 125

att ggt ccc cgg agc ccc tac ggc ttt agg cat gga gcc agc gtc aac 432
Ile Gly Pro Arg Ser Pro Tyr Gly Phe Arg His Gly Ala Ser Val Asn
130 135 140

tac gat gac tac taa 447
Tyr Asp Asp Tyr
145

<210> 21
<211> 148
<212> PRT
<213> Homo sapiens

<400> 21
Met Ala Ala Ser Pro Ala Arg Pro Ala Val Leu Ala Leu Thr Gly Leu
1 5 10 15

Ala Leu Leu Leu Leu Leu Cys Trp Gly Pro Gly Gly Ile Ser Gly Asn
20 25 30

Lys Leu Lys Leu Met Leu Gln Lys Arg Glu Ala Pro Val Pro Thr Lys
35 40 45

Thr Lys Val Ala Val Asp Glu Asn Lys Ala Lys Glu Phe Leu Gly Ser
50 55 60

Leu Lys Arg Gln Lys Arg Gln Leu Trp Asp Arg Thr Arg Pro Glu Val
65 70 75 80

Gln Gln Trp Tyr Gln Gln Phe Leu Tyr Met Gly Phe Asp Glu Ala Lys
85 90 95

Phe Glu Asp Asp Ile Thr Tyr Trp Leu Asn Arg Asp Arg Asn Gly His
100 105 110

Glu Tyr Tyr Gly Asp Tyr Tyr Gln Arg His Tyr Asp Glu Asp Ser Ala
115 120 125

Ile Gly Pro Arg Ser Pro Tyr Gly Phe Arg His Gly Ala Ser Val Asn
130 135 140

Tyr Asp Asp Tyr
145

<210> 22
<211> 3132
<212> DNA
<213> Mus musculus

<220>
<221> CDS
<222> (630)..(1358)

<400> 22
gggggtctgc atctccatcg gaaagtgcgc tggccacatc ccttcggcct ccgggcagtg 60

ttctgtctcc cttagctcag gcagcgagaa acttcagctg tgaagtggtg gtggagagag 120

ccctgggagc agcgactgga cccggacacc aagaagagag tggacgcgcc cctcgactag 180

gaatcgctct cgcaggcgga gacccagcat ctcagcgcct gcggtcgcgc ttgcccggcc 240

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gcgcgctttt gctaggcgcc gccagccccg aaggaccctc ggggtccgcg gacccttctg     300

cagccggcgg aatcctaaag ctgccaagag ctcccggcgg gtgtcggcaa actttttccg     360

agcccacgtg ctgaccaaac agcccggctc gcttccagag cctggcatgg agcgccgcgc     420

ctaggcacgc cgtgcagccc gagagacgcg agcgcacggt tcaccgtgga gggagagatg     480

ctcatcgagc caaattgatc attgcagccc cagggcagtg acatctgtct ctgagtcctc     540

cctaggagcg cgacccgcac tgtctccttc caggagcccg tcatttcctc gacttttgag     600

aggtgtctct ccccagcccg accgtccag atg cgt ttt tgc ctc ttc tca ttt      653
                                Met Arg Phe Cys Leu Phe Ser Phe
                                1               5

gcc ctc atc att ctg aac tgt atg gat tac agc cag tgc caa ggc aac      701
Ala Leu Ile Ile Leu Asn Cys Met Asp Tyr Ser Gln Cys Gln Gly Asn
    10                  15                  20

cga tgg aga cgc aat aag cga gct agt tat gta tca aat ccc att tgc      749
Arg Trp Arg Arg Asn Lys Arg Ala Ser Tyr Val Ser Asn Pro Ile Cys
25                  30                  35                  40

aag ggt tgt ttg tct tgt tcg aag gac aat ggt tgc agc cga tgt caa      797
Lys Gly Cys Leu Ser Cys Ser Lys Asp Asn Gly Cys Ser Arg Cys Gln
                45                  50                  55

cag aag ttg ttc ttt ttc ctt cga aga gaa gga atg cgt cag tat gga      845
Gln Lys Leu Phe Phe Phe Leu Arg Arg Glu Gly Met Arg Gln Tyr Gly
            60                  65                  70

gag tgc ctg cat tcc tgc cca tca ggg tat tat gga cac cga gcc cca      893
Glu Cys Leu His Ser Cys Pro Ser Gly Tyr Tyr Gly His Arg Ala Pro
        75                  80                  85

gat atg aac aga tgt gca cga tgc aga ata gaa aac tgt gat tct tgc      941
Asp Met Asn Arg Cys Ala Arg Cys Arg Ile Glu Asn Cys Asp Ser Cys
    90                  95                  100

ttt agc aaa gac ttt tgt acg aag tgc aaa gta ggc ttt tat ttg cat      989
Phe Ser Lys Asp Phe Cys Thr Lys Cys Lys Val Gly Phe Tyr Leu His
105                 110                 115                 120

aga ggc cgc tgc ttt gat gaa tgt cca gat ggt ttt gca ccg tta gat     1037
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Arg Gly Arg Cys Phe Asp Glu Cys Pro Asp Gly Phe Ala Pro Leu Asp
                125                 130                 135

gag act atg gaa tgt gta gaa ggt tgt gaa gtt ggt cat tgg agc gaa      1085
Glu Thr Met Glu Cys Val Glu Gly Cys Glu Val Gly His Trp Ser Glu
            140                 145                 150

tgg gga acg tgt agc aga aac aac cgc acg tgt gga ttt aaa tgg ggt      1133
Trp Gly Thr Cys Ser Arg Asn Asn Arg Thr Cys Gly Phe Lys Trp Gly
        155                 160                 165

ctg gaa acc aga aca cgg cag att gtt aaa aag cca gca aaa gac aca      1181
Leu Glu Thr Arg Thr Arg Gln Ile Val Lys Lys Pro Ala Lys Asp Thr
    170                 175                 180

ata cca tgt ccg acc att gcg gag tcc agg aga tgc aag atg gcc atg      1229
Ile Pro Cys Pro Thr Ile Ala Glu Ser Arg Arg Cys Lys Met Ala Met
185                 190                 195                 200

agg cac tgt cca gga gga aag aga aca cca aag gca aaa gag aag aga      1277
Arg His Cys Pro Gly Gly Lys Arg Thr Pro Lys Ala Lys Glu Lys Arg
                205                 210                 215

aac aag aag aag agg cgg aag ctg att gag aga gcc caa gag cag cac      1325
Asn Lys Lys Lys Arg Arg Lys Leu Ile Glu Arg Ala Gln Glu Gln His
            220                 225                 230

agc gtc ttc ctc gct aca gac aga gtg aac caa taaaatacaa gaaatagctg    1378
Ser Val Phe Leu Ala Thr Asp Arg Val Asn Gln
        235                 240

gggcattttg aggttttctg ttttgtttat gttgttgttt tgcaaaagtg cacaaagcta    1438

ctctccagtc cacactggtg gacagcattc ctgatcctct gaccagtatc cattttcagt    1498

aatgctgcag agggaggtgc ccaagcatgg actcagcgtt atttatgctt tgattggaat    1558

ctggggcctg tgatggcagg agcttgttga gctgagtcag cgggagctga tgcatctgta    1618

ctcttgtgat gagcacagtg tgtcataaga acctgtccct ggcacggtgg acccacagga    1678

ggcacaaggc tgtagatcac caccagagaa tgcacctgtg cctattttga tggatggcaa    1738

tgctaagcaa gcaagcactg ttcacttgtg actttcattt ctcacactgt gcactgtcaa    1798

agacaaatgt gcatggaaaa atgtttagtg tcacctcatg gcgttctcag catcagtgac    1858
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cttcaaacgg tcctacaatg agactgtgtt ctagctaggg gtatgctgtg gaaattcctg 1918

ctacatttca tcttagtgct aacatgtaca gattctgctg cgctacattc aaagctcatt 1978

actgtatatt tatgctttct ctgtgtaaca agttatacct gataagatgt cactttgttt 2038

ctagtgattc ttaaccatgg tctggtacat ggctattcta gttttggaaa ttaacaagtg 2098

ttttgttgcc tcttgttttc ttttgttcct atcatttttg gcgggggttg ggtgggcttg 2158

attctaaccg taagtatagg ataagctagt tttgtatata gagtcaaatg actgatgtca 2218

gaggatcagt gctgatagaa cttccccagt tcatgtcacg atacacacag agagaaagca 2278

gcatgaggca tcttgccatc agaagccaaa tttcttttga gtcccaaaat tgatgactta 2338

tgaaatatag ctgaaaacaa gatttgggtg tagttacttg tatttattat acaatttcca 2398

attacatttt ttttcaaact caaaataacc catgactttg agtgataggt cacttggcaa 2458

tgttcttgaa ttactgggga agctgttgtc actaagataa tgagagagaa aatagaatgg 2518

cttcgcccaa gtgagagcca catcttacat ttctctgttg aatcggaatc aactatatta 2578

gaacagaagc ctgatagaag ctttctagtt aacacacaca aggccatggt ttcaaaaaca 2638

tctttgtccc cttaggtcag tttgtcctta gattatgaat tggcaggttc taattgcatt 2698

atttccctgg ctgatccagg aaaaagttag aacaaaataa gttgcatagt tttgaggaaa 2758

catccaaagc aaggcgaagc ctttccttgc cttgcattgg caaaactacc tctttagcat 2818

ttatgttgat tcagaaacat cttgctgata tgtgtagatg ttttaagctt cattgtgaaa 2878

atattgatgc aagataagcc atatatgaat gttgtattca actttagggc ttgaaattaa 2938

tcctaaagtg ttcacctctc tccatgtcta tttacactct gttcctattt actaagaggg 2998

taggggtctc cttaatatca tacttcattg ttaataagtc aatgcttgtt atgtttcttg 3058

gctgttgttt ttgtgcatta aaaactcaaa attggaaaaa aaaaaaaaaa aaaaaaaaaa 3118

aaaaaaaaaa aaaa 3132

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<211> 243
<212> PRT
<213> Mus musculus

<400> 23

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Met Arg Phe Cys Leu Phe Ser Phe Ala Leu Ile Ile Leu Asn Cys Met
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Asp Tyr Ser Gln Cys Gln Gly Asn Arg Trp Arg Arg Asn Lys Arg Ala
            20                  25                  30

Ser Tyr Val Ser Asn Pro Ile Cys Lys Gly Cys Leu Ser Cys Ser Lys
        35                  40                  45

Asp Asn Gly Cys Ser Arg Cys Gln Gln Lys Leu Phe Phe Phe Leu Arg
    50                  55                  60

Arg Glu Gly Met Arg Gln Tyr Gly Glu Cys Leu His Ser Cys Pro Ser
65                  70                  75                  80

Gly Tyr Tyr Gly His Arg Ala Pro Asp Met Asn Arg Cys Ala Arg Cys
                85                  90                  95

Arg Ile Glu Asn Cys Asp Ser Cys Phe Ser Lys Asp Phe Cys Thr Lys
            100                 105                 110

Cys Lys Val Gly Phe Tyr Leu His Arg Gly Arg Cys Phe Asp Glu Cys
        115                 120                 125

Pro Asp Gly Phe Ala Pro Leu Asp Glu Thr Met Glu Cys Val Glu Gly
    130                 135                 140

Cys Glu Val Gly His Trp Ser Glu Trp Gly Thr Cys Ser Arg Asn Asn
145                 150                 155                 160
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Arg Thr Cys Gly Phe Lys Trp Gly Leu Glu Thr Arg Thr Arg Gln Ile
165 170 175

Val Lys Lys Pro Ala Lys Asp Thr Ile Pro Cys Pro Thr Ile Ala Glu
180 185 190

Ser Arg Arg Cys Lys Met Ala Met Arg His Cys Pro Gly Gly Lys Arg
195 200 205

Thr Pro Lys Ala Lys Glu Lys Arg Asn Lys Lys Lys Arg Arg Lys Leu
210 215 220

Ile Glu Arg Ala Gln Glu Gln His Ser Val Phe Leu Ala Thr Asp Arg
225 230 235 240

Val Asn Gln

<210> 24
<211> 843
<212> DNA
<213> Mus musculus

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<400> 24
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caacctgggc g atg ccc cgc tac gag ttg gct ttg att ctg aaa gcc atg 170
Met Pro Arg Tyr Glu Leu Ala Leu Ile Leu Lys Ala Met
1 5 10

cgg cgg cca gag acc gct gct gct ttg aaa cgt aca ata gaa tcc ctg 218
Arg Arg Pro Glu Thr Ala Ala Ala Leu Lys Arg Thr Ile Glu Ser Leu
15 20 25

atg gac cga gga gcc ata gtg agg aac ttg gaa agc ctg ggt gag cgt 266
Met Asp Arg Gly Ala Ile Val Arg Asn Leu Glu Ser Leu Gly Glu Arg
30 35 40 45

gcg ctc ccc tac agg atc tcg agt cac agc cag cag cac agc cga gga 314
Ala Leu Pro Tyr Arg Ile Ser Ser His Ser Gln Gln His Ser Arg Gly
50 55 60

ggg tat ttc ctg gtg gat ttt tat gct ccg aca agt gct gtg gag aac 362
Gly Tyr Phe Leu Val Asp Phe Tyr Ala Pro Thr Ser Ala Val Glu Asn
65 70 75

ata ctg gaa cac ttg gcg cga gac att gac gtg gtt aga cca aat att 410
Ile Leu Glu His Leu Ala Arg Asp Ile Asp Val Val Arg Pro Asn Ile
80 85 90

gtg aaa cac cct ctg acc cag gaa gta aaa gag tgt gac ggc ata gtc 458
Val Lys His Pro Leu Thr Gln Glu Val Lys Glu Cys Asp Gly Ile Val
95 100 105

cca gtc cca ctt gaa gaa aaa ctg tat tca aca aag agg agg aag aag 506
Pro Val Pro Leu Glu Glu Lys Leu Tyr Ser Thr Lys Arg Arg Lys Lys
110 115 120 125

tgagaagatt caccagattc tggccttata tttaatccta agggcactat gggtgctgct 566

aggttgttgt ctaggatact ttagcccatg accattttgc tgcaggaggt agaaactgct 626

ggccgagacc tgccctgatg tctctgctga gatttcatcc cacttgtggg gtttgtcggg 686

agtgggggtg ttcacagtac cactgtagcg tttccaagag caaaatgttt gtcattcaca 746

cttggttgtc ttgcaagcct atatggaaca ctgggagcag agtaataaac atgactttat 806

caacactgga aaaaaaaaaa aaaaaaaaaa aaaaaaa 843

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<211> 125
<212> PRT
<213> Mus musculus

<400> 25
Met Pro Arg Tyr Glu Leu Ala Leu Ile Leu Lys Ala Met Arg Arg Pro
1 5 10 15

Glu Thr Ala Ala Ala Leu Lys Arg Thr Ile Glu Ser Leu Met Asp Arg
20 25 30

Gly Ala Ile Val Arg Asn Leu Glu Ser Leu Gly Glu Arg Ala Leu Pro
35 40 45

Tyr Arg Ile Ser Ser His Ser Gln Gln His Ser Arg Gly Gly Tyr Phe
50 55 60

Leu Val Asp Phe Tyr Ala Pro Thr Ser Ala Val Glu Asn Ile Leu Glu
65 70 75 80

His Leu Ala Arg Asp Ile Asp Val Val Arg Pro Asn Ile Val Lys His
85 90 95

Pro Leu Thr Gln Glu Val Lys Glu Cys Asp Gly Ile Val Pro Val Pro
100 105 110

Leu Glu Glu Lys Leu Tyr Ser Thr Lys Arg Arg Lys Lys
115 120 125

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<211> 2490
<212> DNA
<213> Mus musculus

<220>
<221> CDS
<222> (1)..(2487)

<400> 26
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Met Lys Pro Pro Gly Ser Ile Ser Arg Arg Pro Thr Leu Thr Gly Cys
1 5 10 15

agc ctt ccc ggc gcc tcc tgc ggc ccc ggc cgc tgc ccc gcc ggc ccg 96
Ser Leu Pro Gly Ala Ser Cys Gly Pro Gly Arg Cys Pro Ala Gly Pro
20 25 30

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gtg ccg gcc cgc gcg ccg ccc tgc cgc ctg ctc ctc gtc ctt ctc ctg     144
Val Pro Ala Arg Ala Pro Pro Cys Arg Leu Leu Leu Val Leu Leu Leu
        35                  40                  45

cta cct gcg ctc gcc acc tca tcc cgg ccc cgt gcc cgg ggg gcc gct     192
Leu Pro Ala Leu Ala Thr Ser Ser Arg Pro Arg Ala Arg Gly Ala Ala
    50                  55                  60

gcg ccc agc gct ccg cac tgg aat gaa act gca gaa aaa acc ctg gga     240
Ala Pro Ser Ala Pro His Trp Asn Glu Thr Ala Glu Lys Thr Leu Gly
65                  70                  75                  80

gtc ctg gca gat gaa gac aac aca ttg caa caa aat agc agc agc aga     288
Val Leu Ala Asp Glu Asp Asn Thr Leu Gln Gln Asn Ser Ser Ser Arg
                85                  90                  95

aat acc agc tac agc agt gca gtg caa aaa gaa atc aca ctg cct tca     336
Asn Thr Ser Tyr Ser Ser Ala Val Gln Lys Glu Ile Thr Leu Pro Ser
            100                 105                 110

aga ctg gtg tat tac atc aac cag gac tca gaa agc ccc tat cat gtt     384
Arg Leu Val Tyr Tyr Ile Asn Gln Asp Ser Glu Ser Pro Tyr His Val
        115                 120                 125

ctt gac aca aag gcc aga cac caa cag aaa cac aat aag gct gtg cat     432
Leu Asp Thr Lys Ala Arg His Gln Gln Lys His Asn Lys Ala Val His
    130                 135                 140

ctg gcc cag gca agc ttc cag atc gaa gct ttc ggc tcc aag ttc att     480
Leu Ala Gln Ala Ser Phe Gln Ile Glu Ala Phe Gly Ser Lys Phe Ile
145                 150                 155                 160

ctt gac ctc aca ctg aac aat ggt ttg cta tct tct gac tac gtg gag     528
Leu Asp Leu Thr Leu Asn Asn Gly Leu Leu Ser Ser Asp Tyr Val Glu
                165                 170                 175

atc cac tat gaa gac ggg aag cag atg tac tct aag ggt gga gag cac     576
Ile His Tyr Glu Asp Gly Lys Gln Met Tyr Ser Lys Gly Gly Glu His
            180                 185                 190

tgt tac tac cac gga agc atc aga ggc gtc aag gat tcc agg gtg gct     624
Cys Tyr Tyr His Gly Ser Ile Arg Gly Val Lys Asp Ser Arg Val Ala
        195                 200                 205

cta tcg acc tgc aat gga ctc cat ggc atg ttt gag gat gac acc ttt     672
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Leu Ser Thr Cys Asn Gly Leu His Gly Met Phe Glu Asp Asp Thr Phe
    210                 215                 220

gtg tat atg ata gag cct ctg gaa ctg act gat gat gag aaa agc aca      720
Val Tyr Met Ile Glu Pro Leu Glu Leu Thr Asp Asp Glu Lys Ser Thr
225                 230                 235                 240

ggc cga ccg cac ata atc cag aaa acc ttg gca gga cag tat tct aag      768
Gly Arg Pro His Ile Ile Gln Lys Thr Leu Ala Gly Gln Tyr Ser Lys
                245                 250                 255

cag atg aag aat ctc agc aca gat ggc agt gac cag tgg cct ttg cta      816
Gln Met Lys Asn Leu Ser Thr Asp Gly Ser Asp Gln Trp Pro Leu Leu
            260                 265                 270

cct gaa tta caa tgg ctg aga aga agg aaa aga gcg gtc aat cca tct      864
Pro Glu Leu Gln Trp Leu Arg Arg Arg Lys Arg Ala Val Asn Pro Ser
        275                 280                 285

cgt ggt gtg ttt gaa gaa atg aag tat ttg gag ctt atg att gtt aat      912
Arg Gly Val Phe Glu Glu Met Lys Tyr Leu Glu Leu Met Ile Val Asn
    290                 295                 300

gat cac aag acg tat aag aag cac cgc tct tct cac gcg cat acc aac      960
Asp His Lys Thr Tyr Lys Lys His Arg Ser Ser His Ala His Thr Asn
305                 310                 315                 320

aac ttc gca aag tct gtg gtc aac ctt gta gat tct att tac aag gaa     1008
Asn Phe Ala Lys Ser Val Val Asn Leu Val Asp Ser Ile Tyr Lys Glu
                325                 330                 335

cag ctc aac acc agg gtt gtc ctg gtg gct gtc gag acc tgg acc gag     1056
Gln Leu Asn Thr Arg Val Val Leu Val Ala Val Glu Thr Trp Thr Glu
            340                 345                 350

aag gat cac att gac atc acc atc aac ccc gtg cag atg cta cat gac     1104
Lys Asp His Ile Asp Ile Thr Ile Asn Pro Val Gln Met Leu His Asp
        355                 360                 365

ttc tcc aag tac cgg cag cga atc aaa cag cac gct gac gcg gtc cac     1152
Phe Ser Lys Tyr Arg Gln Arg Ile Lys Gln His Ala Asp Ala Val His
    370                 375                 380

ctc atc tcg cgc gtg aca ttc cat tat aag aga agc agt ctg agt tac     1200
Leu Ile Ser Arg Val Thr Phe His Tyr Lys Arg Ser Ser Leu Ser Tyr
385                 390                 395                 400
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ttt gga ggc gtg tgt tct cga ata aga ggg gtt ggt gtg aat gag tat 1248
Phe Gly Gly Val Cys Ser Arg Ile Arg Gly Val Gly Val Asn Glu Tyr
405 410 415

ggt ctt cca atg gcg gtg gca caa gta tta tca cag agc ctg gct caa 1296
Gly Leu Pro Met Ala Val Ala Gln Val Leu Ser Gln Ser Leu Ala Gln
420 425 430

aac ctt gga atc cag tgg gaa cct tcg agc agg aag cca aaa tgt gaa 1344
Asn Leu Gly Ile Gln Trp Glu Pro Ser Ser Arg Lys Pro Lys Cys Glu
435 440 445

tgc ata gag tcc tgg ggc ggc tgc atc atg gaa gaa aca ggg gtg tcc 1392
Cys Ile Glu Ser Trp Gly Gly Cys Ile Met Glu Glu Thr Gly Val Ser
450 455 460

cac tct cga aag ttc tca aag tgc agc att ttg gag tac aga gac ttt 1440
His Ser Arg Lys Phe Ser Lys Cys Ser Ile Leu Glu Tyr Arg Asp Phe
465 470 475 480

tta cag aga ggt ggc gga gca tgt ctt ttc aat agg cca act aag ctg 1488
Leu Gln Arg Gly Gly Gly Ala Cys Leu Phe Asn Arg Pro Thr Lys Leu
485 490 495

ttt gag ccc acg gaa tgt gga aat gga tat gtg gag gcc ggg gag gaa 1536
Phe Glu Pro Thr Glu Cys Gly Asn Gly Tyr Val Glu Ala Gly Glu Glu
500 505 510

tgc gac tgt ggt ttc cat gtg gaa tgc tat gga gtt tgc tgt aag aag 1584
Cys Asp Cys Gly Phe His Val Glu Cys Tyr Gly Val Cys Cys Lys Lys
515 520 525

tgt tcg ctc tcc aat ggg gcc cac tgc agt gac ggc ccc tgc tgt aac 1632
Cys Ser Leu Ser Asn Gly Ala His Cys Ser Asp Gly Pro Cys Cys Asn
530 535 540

aac acc tca tgt ctt ttt cag tca cga ggg tat gaa tgt cgg gat gcc 1680
Asn Thr Ser Cys Leu Phe Gln Ser Arg Gly Tyr Glu Cys Arg Asp Ala
545 550 555 560

gta aac agc tgt gat atc acc gag tac tgc act gga gac tct ggc cag 1728
Val Asn Ser Cys Asp Ile Thr Glu Tyr Cys Thr Gly Asp Ser Gly Gln
565 570 575

tgc cca ccg aac ctc cat aaa caa gat ggc tat agc tgc aat caa aat 1776

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Cys Pro Pro Asn Leu His Lys Gln Asp Gly Tyr Ser Cys Asn Gln Asn
            580                 585                 590

cag ggt cgc tgc tac aat ggc gag tgc aag aca agg gac aat caa tgc      1824
Gln Gly Arg Cys Tyr Asn Gly Glu Cys Lys Thr Arg Asp Asn Gln Cys
        595                 600                 605

cag tac atc tgg ggg aca aag gct gcg ggg tca gac aag ttc tgc tat      1872
Gln Tyr Ile Trp Gly Thr Lys Ala Ala Gly Ser Asp Lys Phe Cys Tyr
    610                 615                 620

gaa aag ctg aac acg gaa ggc acc gag aag ggc aat tgt gga aag gat      1920
Glu Lys Leu Asn Thr Glu Gly Thr Glu Lys Gly Asn Cys Gly Lys Asp
625                 630                 635                 640

gga gac cgg tgg atc ccg tgc agc aag cat gat gtg ttc tgt gga ttt      1968
Gly Asp Arg Trp Ile Pro Cys Ser Lys His Asp Val Phe Cys Gly Phe
                645                 650                 655

ctg ctt tgc acc aat ctt acc cga gct cca cgt atc ggt caa ctt caa      2016
Leu Leu Cys Thr Asn Leu Thr Arg Ala Pro Arg Ile Gly Gln Leu Gln
            660                 665                 670

gga gag atc atc ccg act tcc ttc tat cat caa ggc cga gtg att gac      2064
Gly Glu Ile Ile Pro Thr Ser Phe Tyr His Gln Gly Arg Val Ile Asp
        675                 680                 685

tgc agt ggt gct cat gta gtt tta gac gat gat aca gac gtg ggt tac      2112
Cys Ser Gly Ala His Val Val Leu Asp Asp Asp Thr Asp Val Gly Tyr
    690                 695                 700

gtt gaa gat ggg act ccg tgt ggc ccc tcc atg atg tgc tta gat cgg      2160
Val Glu Asp Gly Thr Pro Cys Gly Pro Ser Met Met Cys Leu Asp Arg
705                 710                 715                 720

aag tgc cta cag att caa gcc ctg aat atg agc agc tgc cca ctt gac      2208
Lys Cys Leu Gln Ile Gln Ala Leu Asn Met Ser Ser Cys Pro Leu Asp
                725                 730                 735

tca agg ggt aaa gtc tgc tcc ggc cac ggg gtg tgt agc aac gaa gcc      2256
Ser Arg Gly Lys Val Cys Ser Gly His Gly Val Cys Ser Asn Glu Ala
            740                 745                 750

acc tgc atc tgt gat ttc act tgg gca ggc aca gac tgc agc atc cgg      2304
Thr Cys Ile Cys Asp Phe Thr Trp Ala Gly Thr Asp Cys Ser Ile Arg
        755                 760                 765
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gat cca gtt cgg aac ccc aac ccc cct aag gat gaa ggc cct aag ggt      2352
Asp Pro Val Arg Asn Pro Asn Pro Pro Lys Asp Glu Gly Pro Lys Gly
    770                 775                 780

cct agc gcc acc aat ctc ata ata ggc tcc atc gct ggt gcc atc ctg      2400
Pro Ser Ala Thr Asn Leu Ile Ile Gly Ser Ile Ala Gly Ala Ile Leu
785                 790                 795                 800

gta gca gct att gtc ctt ggg ggc aca ggc tgg gga ttt aaa aac gtc      2448
Val Ala Ala Ile Val Leu Gly Gly Thr Gly Trp Gly Phe Lys Asn Val
                805                 810                 815

aag aag agg aga ttc gat ccc act cag caa ggc ccc atc tga              2490
Lys Lys Arg Arg Phe Asp Pro Thr Gln Gln Gly Pro Ile
            820                 825


<210>  27
<211>  829
<212>  PRT
<213>  Mus musculus

<400>  27
Met Lys Pro Pro Gly Ser Ile Ser Arg Arg Pro Thr Leu Thr Gly Cys
1               5                   10                  15


Ser Leu Pro Gly Ala Ser Cys Gly Pro Gly Arg Cys Pro Ala Gly Pro
            20                  25                  30


Val Pro Ala Arg Ala Pro Pro Cys Arg Leu Leu Leu Val Leu Leu Leu
        35                  40                  45


Leu Pro Ala Leu Ala Thr Ser Ser Arg Pro Arg Ala Arg Gly Ala Ala
    50                  55                  60


Ala Pro Ser Ala Pro His Trp Asn Glu Thr Ala Glu Lys Thr Leu Gly
65                  70                  75                  80


Val Leu Ala Asp Glu Asp Asn Thr Leu Gln Gln Asn Ser Ser Ser Arg
                85                  90                  95
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Asn Thr Ser Tyr Ser Ser Ala Val Gln Lys Glu Ile Thr Leu Pro Ser
100 105 110

Arg Leu Val Tyr Tyr Ile Asn Gln Asp Ser Glu Ser Pro Tyr His Val
115 120 125

Leu Asp Thr Lys Ala Arg His Gln Gln Lys His Asn Lys Ala Val His
130 135 140

Leu Ala Gln Ala Ser Phe Gln Ile Glu Ala Phe Gly Ser Lys Phe Ile
145 150 155 160

Leu Asp Leu Thr Leu Asn Asn Gly Leu Leu Ser Ser Asp Tyr Val Glu
165 170 175

Ile His Tyr Glu Asp Gly Lys Gln Met Tyr Ser Lys Gly Gly Glu His
180 185 190

Cys Tyr Tyr His Gly Ser Ile Arg Gly Val Lys Asp Ser Arg Val Ala
195 200 205

Leu Ser Thr Cys Asn Gly Leu His Gly Met Phe Glu Asp Asp Thr Phe
210 215 220

Val Tyr Met Ile Glu Pro Leu Glu Leu Thr Asp Asp Glu Lys Ser Thr
225 230 235 240

Gly Arg Pro His Ile Ile Gln Lys Thr Leu Ala Gly Gln Tyr Ser Lys
245 250 255

Gln Met Lys Asn Leu Ser Thr Asp Gly Ser Asp Gln Trp Pro Leu Leu
260 265 270

Pro Glu Leu Gln Trp Leu Arg Arg Arg Lys Arg Ala Val Asn Pro Ser
275 280 285

Arg Gly Val Phe Glu Glu Met Lys Tyr Leu Glu Leu Met Ile Val Asn
290 295 300

Asp His Lys Thr Tyr Lys Lys His Arg Ser Ser His Ala His Thr Asn
305 310 315 320

Asn Phe Ala Lys Ser Val Val Asn Leu Val Asp Ser Ile Tyr Lys Glu
325 330 335

Gln Leu Asn Thr Arg Val Val Leu Val Ala Val Glu Thr Trp Thr Glu
340 345 350

Lys Asp His Ile Asp Ile Thr Ile Asn Pro Val Gln Met Leu His Asp
355 360 365

Phe Ser Lys Tyr Arg Gln Arg Ile Lys Gln His Ala Asp Ala Val His
370 375 380

Leu Ile Ser Arg Val Thr Phe His Tyr Lys Arg Ser Ser Leu Ser Tyr
385 390 395 400

Phe Gly Gly Val Cys Ser Arg Ile Arg Gly Val Gly Val Asn Glu Tyr
405 410 415

Gly Leu Pro Met Ala Val Ala Gln Val Leu Ser Gln Ser Leu Ala Gln
420 425 430

Asn Leu Gly Ile Gln Trp Glu Pro Ser Ser Arg Lys Pro Lys Cys Glu
435 440 445

Cys Ile Glu Ser Trp Gly Gly Cys Ile Met Glu Glu Thr Gly Val Ser
450 455 460

His Ser Arg Lys Phe Ser Lys Cys Ser Ile Leu Glu Tyr Arg Asp Phe
465 470 475 480

Leu Gln Arg Gly Gly Gly Ala Cys Leu Phe Asn Arg Pro Thr Lys Leu
485 490 495

Phe Glu Pro Thr Glu Cys Gly Asn Gly Tyr Val Glu Ala Gly Glu Glu
500 505 510

Cys Asp Cys Gly Phe His Val Glu Cys Tyr Gly Val Cys Cys Lys Lys
515 520 525

Cys Ser Leu Ser Asn Gly Ala His Cys Ser Asp Gly Pro Cys Cys Asn
530 535 540

Asn Thr Ser Cys Leu Phe Gln Ser Arg Gly Tyr Glu Cys Arg Asp Ala
545 550 555 560

Val Asn Ser Cys Asp Ile Thr Glu Tyr Cys Thr Gly Asp Ser Gly Gln
565 570 575

Cys Pro Pro Asn Leu His Lys Gln Asp Gly Tyr Ser Cys Asn Gln Asn
580 585 590

Gln Gly Arg Cys Tyr Asn Gly Glu Cys Lys Thr Arg Asp Asn Gln Cys
595 600 605

Gln Tyr Ile Trp Gly Thr Lys Ala Ala Gly Ser Asp Lys Phe Cys Tyr
610 615 620

Glu Lys Leu Asn Thr Glu Gly Thr Glu Lys Gly Asn Cys Gly Lys Asp
625 630 635 640

Gly Asp Arg Trp Ile Pro Cys Ser Lys His Asp Val Phe Cys Gly Phe
645 650 655

Leu Leu Cys Thr Asn Leu Thr Arg Ala Pro Arg Ile Gly Gln Leu Gln
660 665 670

Gly Glu Ile Ile Pro Thr Ser Phe Tyr His Gln Gly Arg Val Ile Asp
675 680 685

Cys Ser Gly Ala His Val Val Leu Asp Asp Asp Thr Asp Val Gly Tyr
690 695 700

Val Glu Asp Gly Thr Pro Cys Gly Pro Ser Met Met Cys Leu Asp Arg
705 710 715 720

Lys Cys Leu Gln Ile Gln Ala Leu Asn Met Ser Ser Cys Pro Leu Asp
725 730 735

Ser Arg Gly Lys Val Cys Ser Gly His Gly Val Cys Ser Asn Glu Ala
740 745 750

Thr Cys Ile Cys Asp Phe Thr Trp Ala Gly Thr Asp Cys Ser Ile Arg
755 760 765

Asp Pro Val Arg Asn Pro Asn Pro Pro Lys Asp Glu Gly Pro Lys Gly
770 775 780

Pro Ser Ala Thr Asn Leu Ile Ile Gly Ser Ile Ala Gly Ala Ile Leu
785 790 795 800

Val Ala Ala Ile Val Leu Gly Gly Thr Gly Trp Gly Phe Lys Asn Val
805 810 815

Lys Lys Arg Arg Phe Asp Pro Thr Gln Gln Gly Pro Ile
820 825

<210> 28
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<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (1)..(2496)

<400> 28
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Met Lys Pro Pro Gly Ser Ser Ser Arg Gln Pro Pro Leu Ala Gly Cys
1               5                   10                  15

agc ctt gcc ggc gct tcc tgc ggc ccc caa cgc ggc ccc gcc ggc tcg      96
Ser Leu Ala Gly Ala Ser Cys Gly Pro Gln Arg Gly Pro Ala Gly Ser
            20                  25                  30

gtg cct gcc agc gcc ccg gcc cgc acg ccg ccc tgc cgc ctg ctt ctc     144
Val Pro Ala Ser Ala Pro Ala Arg Thr Pro Pro Cys Arg Leu Leu Leu
        35                  40                  45

gtc ctt ctc ctg ctg cct ccg ctc gcc gcc tcg tcc cgg ccc cgc gcc     192
Val Leu Leu Leu Leu Pro Pro Leu Ala Ala Ser Ser Arg Pro Arg Ala
    50                  55                  60

tgg ggg gct gct gcg ccc agc gct ccg cat tgg aat gaa act gca gaa     240
Trp Gly Ala Ala Ala Pro Ser Ala Pro His Trp Asn Glu Thr Ala Glu
65                  70                  75                  80

aaa aat ttg gga gtc ctg gca gat gaa gac aat aca ttg caa cag aat     288
Lys Asn Leu Gly Val Leu Ala Asp Glu Asp Asn Thr Leu Gln Gln Asn
                85                  90                  95

agc agc agt aat atc agt tac agc aat gca atg cag aaa gaa atc aca     336
Ser Ser Ser Asn Ile Ser Tyr Ser Asn Ala Met Gln Lys Glu Ile Thr
            100                 105                 110

ctg cct tca aga ctc ata tat tac atc aac caa gac tcg gaa agc cct     384
Leu Pro Ser Arg Leu Ile Tyr Tyr Ile Asn Gln Asp Ser Glu Ser Pro
        115                 120                 125

tat cac gtt ctt gac aca aag gca aga cac cag caa aaa cat aat aag     432
Tyr His Val Leu Asp Thr Lys Ala Arg His Gln Gln Lys His Asn Lys
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130 135 140

gct gtc cat ctg gcc cag gca agc ttc cag att gaa gcc ttc ggc tcc 480
Ala Val His Leu Ala Gln Ala Ser Phe Gln Ile Glu Ala Phe Gly Ser
145 150 155 160

aaa ttc att ctt gac ctc ata ctg aac aat ggt ttg ttg tct tct gat 528
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165 170 175

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(75) Inventors/Applicants *(for US only)*: **NISHIKAWA, Mitsuo** [JP/JP]; C/O KIRIN BEER KABUSHIKI KAISHA, Iyaku Tansaku Kenkyusho, 3, Miyahara-cho, Takasaki-shi, Gunma 370-1295 (JP). **DRMANAC, Radoje T.** [US/US]; 850 East Greenwich Place, Palo Alto, CA 94303 (US). **LABAT, Ivan** [YU/US]; 1006 Asbury Way, Mountain View, CA 94043 (US). **LEE, Juhi** [US/US]; 5631 Spry Common, Fremont, CA 94538 (US). **TANG, Y. Tom** [US/US]; 4230 Ranwick Court, San Jose, CA 95118 (US). **STACHE-CRAIN, Birgit** [DE/US]; 345 South Mary Ave., Sunnyvale, CA 94086 (US).

(74) Agents: **TOYAMA, Tsutomu** et al.; Yokoyama Building 6th floor, 4-10, Higashi Nihonbashi 3-chome, Chuo-ku, Tokyo 103-0004 (JP).

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(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*

(88) Date of publication of the international search report: 30 May 2003

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 02/100898 A3

(54) **Title:** POLYPEPTIDE HAVING AN ACTIVITY TO SUPPORT PROLIFERATION OR SURVIVAL OF HEMATOPOIETIC STEM CELL AND HEMATOPOIETIC PROGENITOR CELL, AND DNA CODING FOR THE SAME

(57) **Abstract:** A gene encoding a polypeptide having an activity to support proliferation or survival of hematopoietic stem cells or hematopoietic progenitor cells is isolated by comparing expressed genes between cells which support proliferation or survival of hematopoietic stem cells or hematopoietic progenitor cells and cells which do not support the proliferation or survival. Proliferation or survival of hematopoietic stem cells or hematopoietic progenitor cells is supported by using stromal cells in which the isolated gene is expressed or a gene product of the isolated gene.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/JP 02/0[illegible]

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07K14/475 C12N15/12 C12N5/06 A61K38/00 C07K16/22
C07K14/47

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07K C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EMBL, EPO-Internal, BIOSIS, WPI Data, PAJ, SEQUENCE SEARCH, EMBASE, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE EMBL 'Online! 9 March 2001 (2001-03-09) "Mus musculus clone MGC:7583 IMAGE:3493553, mRNA complete cds" Database accession no. BC002254 XP002220990 99.8% identity with SEQ ID No 18 in 447 bp overlap the whole document	1-9
X	-& DATABASE SWALL 'Online! 1 June 2001 (2001-06-01) "Hypothetical 17.0 Da protein" Database accession no. Q99LS0 XP002220991 identical to SEQ ID No 19 the whole document --- -/--	1-9

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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"A" document defining the general state of the art which is not considered to be of particular relevance

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"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
7 February 2003	06.03.03

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Weikl, M

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE EMBL 'Online! 26 December 2000 (2000-12-26) "Homo sapiens esophageal cancer related gene 4 protein (ECRG4) mRNA, complete cds" Database accession no. AF325503 XP002220992 cited in the application 100% identity with SEQ ID No 20 in 447 bp overlap and 82.3% identity with SEQ ID No 18 in 440 bp overlap the whole document	1-9
X	-& DATABASE SWALL 'Online! 1 March 2001 (2001-03-01) "Esophageal cancer related gene 4 protein" Database accession no. Q9H1Z8 XP002220993 identical to SEQ ID No 21 and 84.5% identity with SEQ ID No in 148 aa overlap the whole document ---	1-9
Y	MOORE K A ET AL: "HEMATOPOIETIC ACTIVITY OF A STROMAL CELL TRANSMEMBRANE PROTEIN CONTAINING EPIDERMAL GROWTH FACTOR-LIKE REPEAT MOTIFS" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, NATIONAL ACADEMY OF SCIENCE. WASHINGTON, US, vol. 94, April 1997 (1997-04), pages 4011-4016, XP002915979 ISSN: 0027-8424 the whole document ---	1-13
Y	WO 99 03980 A (NAKAHATA TATSUTOSHI ;KIRIN BREWERY (JP)) 28 January 1999 (1999-01-28) abstract ---	1-13
Y	XU M ET AL: "STIMULATION OF HUMAN PRIMITIVE HEMATOPOIESIS BY MURINE AGM-DERIVED STROMAL CELLS" BLOOD, W.B. SAUNDERS, PHILADELPHIA, VA, US, vol. 90, no. 10, 15 November 1997 (1997-11-15), page 483A XP002911189 ISSN: 0006-4971 , last sentence ---	1-13

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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A	MOORE KATERI A ET AL: "In vitro maintenance of highly purified, transplantable hematopoietic stem cells." BLOOD, vol. 89, no. 12, 1997, pages 4337-4347, XP002220989 ISSN: 0006-4971 the whole document ---	1-13
A	EP 0 953 354 A (FUJISAWA PHARMACEUTICAL CO) 3 November 1999 (1999-11-03) example 8 ---	1-13
Y	DAVID G ET AL: "MOLECULAR CLONING OF A PHOSPHATIDYLINOSITOL-ANCHORED MEMBRANE HEPARAN SULFATE PROTEOGLYCAN FROM HUMAN LUNG FIBROBLASTS" JOURNAL OF CELL BIOLOGY, vol. 111, no. 6 PART 2, 1990, pages 3165-3176, XP009005399 ISSN: 0021-9525 the whole document	10-13
Y	-& DATABASE EMBL 'Online! 4 March 1991 (1991-03-04) "Human mRNA for heparan sulfate proteaglycan (glypican)" Database accession no. X54232 XP002230116 the whole document	10-13
Y	-& DATABASE SWALL 'Online! 1 February 1994 (1994-02-01) "Glypican-1 precursor" Database accession no. P35052 XP002230117 cited in the application the whole document ---	10-13
Y	DATABASE EMBL 'Online! 26 September 1999 (1999-09-26) "Mus musculus glypican-1 (Gpc1) mRNA, complete cds" Database accession no. AF185613 XP002230118 cited in the application the whole document	10-13
Y	-& DATABASE SWALL 'Online! 1 May 2000 (2000-05-01) "Glypican-1" Database accession no. Q9QZF2 XP002230119 the whole document --- -/--	10-13

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
Y	SCHOFIELD KAREN P ET AL: "Expression of proteoglycan core proteins in human bone marrow stroma." BIOCHEMICAL JOURNAL, vol. 343, no. 3, 1 November 1999 (1999-11-01), pages 663-668, XP002230115 ISSN: 0264-6021 the whole document	10-13

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [ ] Claims Nos.: –
because they relate to subject matter not required to be searched by this Authority, namely:

2. [X] Claims Nos.: 2b, 11b
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

see FURTHER INFORMATION sheet PCT/ISA/210

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [X] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

1-13 (insofar as they relate to SEQ ID Nos 8-11 and 18-21; i.e. inventions 1 and 4)

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[X] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-13 (insofar as they relate to SEQ ID Nos 18-21)

   claims relating to SCR-5

2. Claims: 1-13 (insofar as they relate to SEQ ID Nos 22 and 23)

   claims relating to SCR-6

3. Claims: 1-13 (insofar as they relate to SEQ ID Nos 24 and 25)

   claims relating to SCR-7

4. Claims: 10-13 (insofar as they relate to SEQ ID Nos 8-11)

   claims relating to SCR-2

5. Claims: 10-13 (insofar as they relate to SEQ ID Nos 12 and 13)

   claims relating to SCR-3

6. Claims: 10-13 (insofar as they relate to SEQ ID Nos 14-17)

   claims relating to SCR-4

7. Claims: 10-13 (insofar as they relate to SEQ ID Nos 26-29)

   claims relating to SCR-8

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 2b, 11b

Present claims 2b and 11b relate among others to DNA which is hybridizable under stringent conditions to a probe prepared from the the nucleotide sequences of the present application. Due to the very unclear wording of the claims, such a probe can be imagined to be prepared in many ways (including possibly even nucleotide exchanges) and is thus neither defined by its length nor by its sequence. A multitude of unrelated DNA molecules can be expected to hybridize to at least one of such probe molecules.
Therefore, present claims 2b and 11b relate among others to DNA molecules only defined by reference to a desirable characteristic or property, namely the activity to support proliferation or survival of hematopoietic stem cells or hematopoietic progenitor cells.

The claims cover all DNA sequences having this characteristic or property, whereas the application provides support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT for only a very limited number of such DNAs namely the sequences defined by the SEQ ID Nos themselves. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Independent of the above reasoning, the claims also lack clarity (Article 6 PCT). An attempt is made to define the DNAs by reference to a result to be achieved. Again, this lack of clarity in the present case is such as to render a meaningful search over the whole of the claimed scope impossible. Consequently, the search has not been out carried out for those parts of claims 2b and 11b which relate to DNA molecules hybridizable to probes prepared from the nucleotide sequences of the present application.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/JP 02/05807

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
WO 9903980	A	28-01-1999	AU	8243798 A	10-02-1999
			WO	9903980 A1	28-01-1999
EP 0953354	A	03-11-1999	EP	0953354 A1	03-11-1999
			US	6495365 B1	17-12-2002
			WO	9806422 A1	19-02-1998